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IRON ABSORPTION IN THE ABSENCE OF BILE[1]

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A number of authors have recently either stated or implied that adequate absorption of iron from the intestine depends upon the presence of bile (2, 3, 4, 5). We have examined the literature carefully and have been unable to find any positive, direct evidence for this view, although there are numerous observations which may be considered to support it indirectly.

Several groups of investigators have reported that anemia follows the exclusion of bile from the intestine (6, 7, 8, 9, 10), and our own experience with a group of bile-fistula dogs confirms this finding. Our animals have been maintained on an adequate diet, without administration of bile, and have remained anemic throughout the period of observation (more than one year in several cases). It did not seem to us that these observations or certain others frequently cited (11, 12, 13) constitute proof that the anemia of the bile-fistula animal results from iron deprivation, nor that it is necessarily a simple secondary anemia, as has been stated (7, 11). Consequently we have investigated the ability of the bile-fistula dog to absorb iron, by a study of plasma iron levels following the oral administration of iron salts. Our results indicate that the absence of bile does not interfere significantly with iron absorption except under special circumstances.

METHODS. "Internal" bile-fistulae were prepared by anastomosis of the gall bladder to the renal pelvis, according to the method of Kapsinow, Engle and Harvey (14). Control animals were taken at random from the incoming supply. All were maintained on the ration currently in use, consisting of a commercial canned dog food plus "Purina Checkers." Except for occasional days on which they were used for fat absorption studies, the bile-fistula animals have been given no bile or bile salts. Several of the animals selected as controls proved to be slightly anemic according to our standards (normal, at least 16 grams of hemoglobin per 100 cc. of blood). All control animals are therefore designated in the tables as "unoperated," rather than "normal." Since iron

[1] A preliminary report of this study has appeared elsewhere (1).

absorption is increased in iron deficiency anemia (15, 16, 17, 18), we felt that the use of spontaneously anemic dogs as controls decreased the possibility of an artificially favorable comparison.

The animals were deprived of one feeding prior to the beginning of an experiment. A basal blood sample was drawn, and the iron salt, in water solution, was given by stomach tube. Ferrous sulfate was used in some of the experiments, but ferrous gluconate[2] was used in the majority, because of evidence of excellent absorbability, and the almost complete absence of gastrointestinal irritation (19). The animals were carefully watched for signs of vomiting or diarrhea. In only one instance, after ferrous sulfate, did vomiting occur, and this experiment was discarded. In a majority of the experiments, as will be noted in the tables, 10 cc. blood samples were drawn at 2 hour intervals, the last one at 6 hours. It has been demonstrated that the peak value of plasma iron following a single dose usually occurs in from 2 to 5 hours (15, 16, 17, 18). All samples were taken from the jugular vein and discharged into 15 cc. centrifuge tubes containing a dry mixture of ammonium and potassium oxalates.

Bile-fistula animal number 3, which has remained in consistently good condition with a hemoglobin level of about 13 grams, was recently sacrificed in the course of another experiment. At autopsy, a dye injection of the biliary tract showed that no bile could have been reaching the intestine. As will be noted in the tables, this animal was one of our best "iron-absorbers."

In all the experiments the hematocrit percentage of the successive samples was recorded, and in many experiments the plasma protein concentration (falling-drop method) was also determined in order to establish that the observed changes in plasma iron could not have resulted from concentration or dilution of the blood.

The difficulties of accurate determination of the inorganic iron of the plasma, and the methods available, have been fully reviewed by Moore (20). We have applied the color reaction first described by Lyons (21) to trichloracetic acid filtrates of plasma according to the procedure given below.

Four cubic centimeter samples of plasma were pipetted into 15 cc. centrifuge tubes, 1 cc. of 0.5N HCl was added to each, and the tubes allowed to stand for 24 hours at room temperature, which is essentially the procedure recommended by Barkan (22), except for our omission of incubation at 37°C. Tompsett (23) has indicated that this procedure converts the ferric iron of the plasma to the ferrous form, releasing it from complexes with phospho-proteins, etc., and rendering it diffusible. Recovery of inorganic iron in trichloracetic acid filtrates is increased by from 7 to 10 per cent as a result. Five cubic centimeters of a 10 per cent solution of trichloracetic acid (reagent grade, redistilled) were added to the acidified plasma. Duplicate 3 cc. portions of the filtrate, in glass-stoppered, graduated, test tubes, were made alkaline to litmus with concentrated, iron-free NH_4OH (about 5 drops) and two drops were added in excess. Two-tenths cubic centimeter of diluted thioglycolic acid (4 cc. thioglycolic acid and 8 cc. concentrated NH_4OH in 50 cc. H_2O) was added to each tube and the volume made up to 5 cc. with distilled water. Color intensity was read by means of a photo-electric colorimeter[3] for which a standard curve had been

[2] The ferrous gluconate used in these experiments was supplied by courtesy of Frederick Stearns and Company, Detroit, Michigan.

[3] The Cenco Photelometer.

prepared, using the green filter. All solutions, including the anticoagulant, have been made up with water redistilled from an all-glass system, and blank determinations including all the reagents have consistently been negative. Multiple tests on the same plasma, and tests for the recovery of added iron indicate that the random, over-all analytical error is less than 10 per cent in the range of concentrations encountered in plasma. The use of trichloracetic acid filtrates has been criticized (20) because a portion of the inorganic iron may be carried down with the precipitate. We believe this error can be disregarded in a comparative study, since it is small, and appears to be constant for a particular plasma.

TABLE 1

Plasma iron following a single dose of ferrous sulfate

0.88 gram $FeSO_4 \cdot 7H_2O$ (177 mgm. ferrous iron) in 100 cc. H_2O, by stomach tube; animals in post-absorptive state

EXPERIMENT	ANIMAL	PLASMA IRON, MICROGRAMS PER CENT						ABSORPTION
		Initial	2 hours	3½ hours	4 hours	5 hours	6 hours	
Bile-fistula								
2/13/40	3	200	467	317				Good
2/20/40	4	167	200	183		200		Poor
2/28/40	1	225	267	242		200		Poor
2/ 3/41	9	183	333		283		266	Good
2/ 3/41	15	133	234		333		266	Good
2/ 3/41	28	167	200		217		208	Poor
Unoperated								
2/13/40	2	359	317	283				None
2/20/40	1	258	442	417		378		Good
2/28/40	2	350	375	358		368		None
2/ 3/41	14	167	358		358		316	Good
2/ 3/41	15	200	358		392		375	Good
Control experiments—no iron fed								
Bile-fistula								
9/19/40	9	225	200		192		167	
9/19/40	3	258	267		200		183	
Unoperated								
9/24/40	11	333			350		317	
9/24/40	12	233			217		217	
10/ 7/40	11	216	204		204		204	
10/ 7/40	13	167	167		167		167	
10/14/40	14	220	201		201		200	
1/ 4/41	15	200	167		167		192	

Moreover, the method has one outstanding advantage in that hemolysis, for which allowance must be made if the plasma is used directly for the determination of inorganic iron, can be disregarded since the ferrihemic acid formed during the HCl treatment is insoluble at pH 6 or below, and is carried down with the precipitate (24).

RESULTS. The results of the first series of experiments are given in table 1. After the withdrawal of the initial blood sample each animal received by stomach tube 0.880 gram $FeSO_4 \cdot 7H_2O$ (177 mgm. ferrous iron) dissolved in 100 cc. of distilled water. It will be noted that all the bile-fistula animals absorbed iron as

indicated by a rise in the plasma level. No rise in plasma iron occurred in two successive tests on one of the unoperated animals, perhaps as a consequence of the extremely high original level (25).

We attach no particular significance to the absolute magnitude of the rise in plasma iron for several reasons. Our method gives us no information as to the rate at which iron is leaving the blood stream. Presumably, absorption is always quantitatively greater than can be accounted for by the increased concentration in the plasma, and could be considerable even in those experiments in which no increase occurred. Arbitrarily fixed sampling times may have caused us to miss the peak values, and the wide differences in original plasma levels make it difficult to choose an index of comparative absorption. Consequently, in this and subsequent tables, we have indicated the magnitude of absorption only roughly, as follows: none, if the rise was less than 20 per cent of the original value, with full recognition of the fact, as stated above, that this does not preclude the possibility that some absorption occurred; poor, if the rise did not exceed 50 per cent of the original value; good, if the rise was 50 per cent or above.

The control experiments included in this table show that in animals under identical conditions there is no tendency for the plasma iron to rise spontaneously, so it seems clear that the absence of bile has little effect on the absorption of iron by a fasting animal from a solution of a ferrous salt.

In view of the results just described, we considered it important to determine, if possible, whether the presence of food in the intestine interfered more seriously with iron absorption in the bile-fistula animals than in the unoperated controls. It appeared obvious from the slow progress of their anemia, as well as its tendency toward occasional spontaneous remissions, that bile-fistula animals must absorb some dietary iron.

The experiments presented in part I of table 2 show that there was no increase in the plasma iron in either type of animal during the 6 hours following a single dose of ferrous gluconate added as the dry powder to the regular diet. There is good absorption in each, however, from a solution of ferrous gluconate given by stomach tube 30 minutes before feeding. We have repeated the first type of experiment numerous times, and in no instance have we detected any increase in plasma iron.

These results indicate that even though some absorption may have occurred, it would be necessary to give much larger quantities of iron in order to establish the fact beyond question by our method. Since the 1 gram dose which we had been using appeared to be about as much as could be added to the ration without making it unpalatable, it seemed preferable to add this amount daily for several days and attempt to build up a high plasma level by accumulation, rather than resort to a larger single dose. The results of a typical series of experiments are shown in part II of table 2. Blood samples were drawn on the day preceding the first dose, the third day of administration, and the day after the fifth and last dose. It is evident from the high iron levels observed on the third and fifth days that good absorption can occur in the bile-fistula dog, since variations of such magnitude do not occur spontaneously. This is shown by basal plasma iron

levels taken from other experiments in which the same animals were used (part III of table 2). It would appear that such variations as do occur in these basal levels are in themselves evidence of absorption of dietary iron, since determinations on successive days of fasting in some 25 animals to date have shown a steady, downward trend. We believe the decrease observed in two of the animals at the fifth day may be accounted for by the poor appetite which became apparent in these animals about the fourth day of the test.

TABLE 2

The influence of food on iron absorption

Part I. 1 gram ferrous gluconate (116 mgm. ferrous iron) mixed with regular food

EXPERIMENT	ANIMAL	PLASMA IRON, MICROGRAMS PER CENT				ABSORPTION
		Initial	2 hours	4 hours	4 hours	
4/3/40	Unoperated no. 3	183	175	133	100	None
4/3/40	Bile-fistula no. 1	200	200	192	158	None
1 gram ferrous gluconate in 100 cc. H_2O by stomach tube, regular food ½ hr. later						
4/3/40	Unoperated no. 4	150	567	408	442	Good
4/3/40	Bile-fistula no. 9	192	550	475	458	Good

Part II. 1 gram ferrous gluconate per day, May 4–8 inclusive, mixed with regular food

ANIMAL	PLASMA IRON, MICROGRAMS PER CENT			ABSORPTION
	5/3/40	5/6/40	5/9/40	
Bile-fistula				
1	167	275	458	Good
3	217	343	225	Good
9	217	300	275	Poor
Unoperated				
4	150	392	475	Good
5	100	217	267	Good

Part III. Basal plasma iron levels, bile-fistula animals, at random over a 1 year period

ANIMAL	PLASMA IRON, MICROGRAMS PER CENT						
	2/13/40	2/28/40	4/3/40	7/11/40	7/24/40	9/19/40	2/3/41
1		225	200	317	200		
3	200			217		258	
9			192	242	233	225	183

Since fat is known to be poorly absorbed in the absence of bile, we wondered whether its presence in the intestine would have any effect on iron absorption in the bile-fistula dog. (The diet used in the experiments just described contained about 5 per cent of fat.) The first five experiments of table 3 show that the addition of a neutral fat (100 cc. of olive oil) to the ferrous gluconate solution definitely inhibits iron absorption during the 6 hour observation period. In only one experiment was there a clear-cut, progressive increase in plasma iron; in another, because of a rise at the sixth hour, absorption is designated as doubtful.

In contrast to this, as the next three experiments show, the presence of olive oil appears not to affect iron absorption in the normal animal. The last five experiments of table 3 show that an inert oil (mineral oil) may possibly interfere with iron absorption in the bile-fistula dog, but does not prevent it.

Attempts to repeat the experiments of part II, table 2, using a diet in which the fat content was raised to about 20 per cent by the addition of corn or cotton-seed oil, have failed to yield definitive results because both unoperated and bile-fistula animals refuse to eat such a mixture with any regularity. We can say, however, that the plasma iron showed fluctuations both above and below the original level in unoperated and bile-fistula animals alike, again suggesting that

TABLE 3

The effect of fat on iron absorption. All animals post-absorptive at beginning of experiment

EXPERIMENT	ANIMAL	PLASMA IRON, MICROGRAMS PER CENT						ABSORPTION
		Initial	2 hours	2½ hours	4 hours	5 hours	6 hours	
1 gram ferrous gluconate in 100 cc. H_2O plus 100 cc. olive oil, by stomach tube								
Bile-fistula								
5/ 3/40	9	217	225		217		167	None
7/11/40	1	317	275		225		183	None
7/11/40	3	217	192		167		233	(Doubtful)
7/24/40	9	233		242		175		None
7/24/40	10	175		217		233		Poor
Normal								
9/19/40	8	208	500		367		275	Good
9/19/40	9	192	400		533		367	Good
9/19/40	10	242	342		442		475	Good
1 gram ferrous gluconate in 100 cc. H_2O plus 100 cc. mineral oil, by stomach tube								
Bile-fistula								
5/ 3/40	1	167	200		167		167	Poor
5/ 3/40	3	217	308		333		342	Good
7/11/40	9	242	292		308		300	Poor
7/11/40	10	200	275		292		242	Good
7/24/40	1	200		242		242		Poor

some absorption must have occurred after the sporadic feedings to which hunger drove them.

Discussion. Our results show that bile-fistula dogs absorb significant amounts of iron from the gastro-intestinal tract when a ferrous compound is given alone, or is added to their regular diet. In fact, under these conditions absorption by the bile-fistula animals is not detectably inferior to that of unoperated controls, as judged by the magnitude and the time relations of the increase in the inorganic iron of the plasma. In the presence of fat, however, absorption proceeds normally in the controls, but cannot be detected during the customary 6 hour observation period in the bile-fistula dogs. It will be noted that mineral oil in similar amounts has only a slight inhibitory effect, indicating that the physical properties of the oil do not account for the interference observed.

Evidence from this laboratory (27) indicates that after a fat meal there is a greater delay in gastric emptying during the latter half of the digestive period in bile-fistula dogs than in unoperated controls. The difference, however, is so slight that one cannot account for the observed differences in iron absorption on the basis of delay in the arrival of food in the intestine of the bile fistula dog. We would conclude that the difference is probably due to the decreased rate of fat absorption in the bile-fistula dog, allowing immobilization of a large portion of the iron in the form of insoluble soaps. Of considerable interest is the fact that a meal comparable to those used in our feeding experiments leaves the stomach at about the same rate in both types of animal. It would appear that under these circumstances the presence of food so delays iron absorption that it is balanced by the rate of migration from the plasma, and therefore no change can be observed in the plasma level. The animal appears to have no difficulty in storing all the iron that can be absorbed from a single large dose, since extension of the observation period to 36 hours gives a similar negative result. On a diet of normal character there seems to be no inhibitory influence peculiar to the bile-fistula animal, and we have shown it to be possible by persistent administration of iron over the course of several days to obtain significant rises in the plasma iron levels of bile-fistula as well as unoperated animals.

While we would not insist that bile-fistula animals are able to absorb iron as well as unoperated animals under all circumstances, we are forced to the conclusion that inadequate absorption cannot be held responsible for their spontaneous, chronic anemia. Aside from the data presented above, we have collected all the post-absorptive plasma iron determinations obtained in the course of this study, and have subjected them to statistical analysis. In an experiment involving determinations on serial samples of blood, only the first was included. The mean of 34 determinations on control animals was 198 micrograms per cent, with a standard deviation of 10.6. The mean of 37 determinations on bile-fistula animals was 204 micrograms per cent, the standard deviation, 6.85. The standard deviation of the difference between the means is 12.65, more than twice the observed difference. Thus the basal values have proved to be identical in bile-fistula and normal dogs. It is generally recognized that iron deficiency anemia is characterized by low plasma iron values (17; note for instance our control animals 4 and 5).

We have presented elsewhere in more detail our reasons for believing that the anemia in the bile-fistula animal is due to lack of the erythrocyte maturation factor (28). It is significant that although Hawkins et al. (12) concluded from their studies that iron absorption is diminished in the bile-fistula dog, they were not convinced that this alone could account for the decreased hemoglobin production which they observed. At that time they suggested the possibility of impaired liver function in the absence of the normal secretion-absorption cycle of the bile salts as an additional factor in its etiology.

SUMMARY

Bile-fistula dogs which have been maintained for long periods without the oral administration of bile remain persistently anemic, yet they absorb iron (as judged

by the rise of the plasma level) in a substantially normal manner if the iron compound is given alone or is added to their standard diet over a period of several days.

Iron given with neutral fat is absorbed so slowly by bile fistula dogs that no change in plasma level occurs over a 36 hour observation period. Control dogs absorb iron well under similar conditions, as judged by the increase in their plasma iron values. Mineral oil given with iron does not prevent a rise in plasma iron in bile fistula animals. Because the changes in gastrointestinal motility that have been observed previously in bile-fistula animals do not seem adequate to account for these effects on iron absorption, we conclude that the prevention of iron absorption by fat in the absence of bile is probably due to the formation of insoluble iron soaps.

The ability of the bile-fistula dog to absorb iron on a low fat diet, together with the normal fasting plasma iron levels of these animals, indicates that the anemia in these dogs is not secondary to iron deficiency.

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STUDIES ON THE BIOLOGICAL UTILIZATION OF ESTERS OF PANTOTHENIC ACID

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Pantothenic acid has been demonstrated to be essential for the maintenance and growth of both microörganisms and laboratory animals. Its growth stimulating effect on yeast and on bacteria served as the basis for the development of quantitative assay methods for this material. Also, its effectiveness in restoring growth and preventing the dermatitis which occurs in chicks maintained on a pantothenic acid free diet has been employed in the development of an assay method with this species. Although no assay methods have been proposed using rats, it has been shown that graded doses of calcium pantothenate produce graded growth responses in rats maintained on purified diets free from pantothenic acid. While such a procedure might not be suitable for assays of pantothenic acid in natural substances, it can be employed to obtain roughly quantitative data on the biological effectiveness of chemically pure substances related to pantothenic acid. The preparation of two esters of pantothenic acid,

(I) $HOCH_2C(CH_3)_2CHOCH_3CO\cdot CONHCH_2CH_2CO_2C_2H_5$

(II) $HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CO_2C_2H_5$

ethyl monoacetyl pantothenate (I) and ethyl pantothenate (II) by Harris, Boyack and Folkers (2) gave the occasion to study their biological activity in comparison with calcium pantothenate.

Microbioassay. The microbiological assays with Lactobacillus casei were carried out according to the method of Pennington, Snell and Williams (5) with the exception that the alkali treated yeast extract (10 mgm. per tube) was supplemented by a mixture of thiamine (10 γ), riboflavin (1 γ), nicotinic acid (10 γ) and pyridoxine (10 γ). The aqueous solutions of the esters (pH 7) were sterilized either by Seitz filtration, by streaming steam at 100°C., or by autoclaving at 15 pounds' pressure for 20 minutes.

Table 1 presents data on the effect of these sterilizing procedures. The activity of ethyl monoacetyl pantothenate sterilized by Seitz filtration was 0.7 per cent and of ethyl pantothenate 6.7 per cent of that of equimolecular amounts of pantothenic acid. A slight increase in activity was found after standing for 6 weeks, indicating some hydrolysis at neutral pH. Steam sterilization as well as autoclaving for 20 minutes at 15 pounds pressure increased the activity considerably, whereas autoclaving with normal sodium hydroxide destroyed the activity of both esters almost completely.

TABLE 1

Effect of various methods of sterilization on the biological activity of ethyl monoacetyl pantothenate and ethyl pantothenate as determined by microbioassay

The activity is expressed in terms of percentage of the activity of equimolecular amounts of pantothenic acid

	SEITZ FILTRATION			STEAM AT 100°C.	AUTOCLAVE AT pH 7	AUTOCLAVE AT pH 14
	Immediately	2 weeks later	4 weeks later			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ethyl monoacetyl pantothenate	0.7	0.67	1.35	2.64	5.05	0.05
Ethyl pantothenate	6.8	6.1	9.9	12.1	25.0	0.7

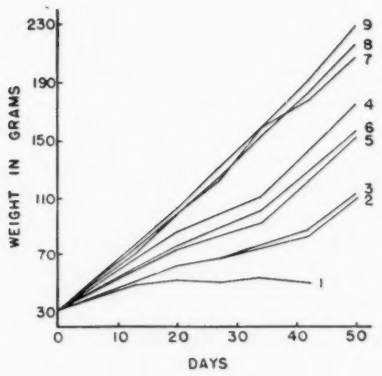

Fig. 1

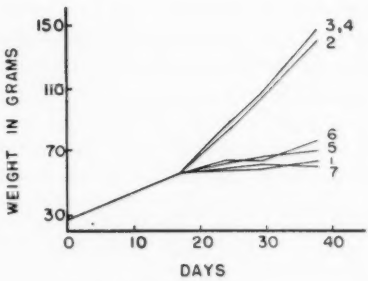

Fig. 2

Fig. 1. The growth promoting effect of equimolecular amounts of ethyl monoacetyl pantothenate, ethyl pantothenate and calcium pantothenate on rats maintained on a basal ration free from pantothenic acid. Each curve represents the average growth rate of 5 animals.

1, no pantothenic acid; *2*, 10γ ethyl pantothenate; *3*, 10γ calcium pantothenate; *4*, 25γ ethyl monoacetyl pantothenate; *5*, 25γ ethyl pantothenate; *6*, 25γ calcium pantothenate; *7*, 100γ ethyl monoacetyl pantothenate; *8*, 100γ ethyl pantothenate; *9*, 100γ calcium pantothenate.

Fig. 2. The response to equimolecular amounts of ethyl monoacetyl pantothenate, ethyl pantothenate and calcium pantothenate in rats depleted for 17 days on a basal ration free from pantothenic acid. Each curve represents the average growth rate of 6 animals.

1, no pantothenic acid; *2*, 100γ ethyl monoacetyl pantothenate (autoclaved at pH 7); *3*, 100γ ethyl pantothenate (autoclaved at pH 7); *4*, 100γ calcium pantothenate (autoclaved at pH 7); *5*, 100γ ethyl monoacetyl pantothenate (autoclaved at pH 14); *6*, 100γ ethyl pantothenate (autoclaved at pH 14); 7, 100γ calcium pantothenate (autoclaved at pH 14).

Animal assay. The assays were conducted in a manner previously described (7) on young albino rats maintained on a diet consisting of dextrose 68 per cent; vitamin-free casein 18 per cent; hydrogenated vegetable oil (crisco) 8 per cent;

salt mixture U.S.P. XI no. 1, 4 per cent; and cod liver oil 2 per cent, supplemented with 0.8 mgm. each of thiamine, riboflavin and pyridoxine, 10 mgm. of nicotinamide, and 100 mgm. of choline chloride per 100 grams of diet. The esters were compared with equimolecular amounts of calcium pantothenate in both preventive and curative tests.

The growth promoting effect of the daily administration of graded doses of the esters as well as of calcium pantothenate in preventive experiments is illustrated in figure 1. Equimolecular amounts of ethyl monoacetyl pantothenate, of ethyl pantothenate and of calcium pantothenate produced comparable increases in the weight of the animals. Furthermore, the condition of the fur and the occurrence of other deficiency symptoms was comparable in groups receiving the smaller amounts of these substances. The animals receiving 100 micrograms of calcium pantothenate or its equivalent of either the two esters grew at approximately the same rate and presented a normal appearance.

The curative effect of the esters of pantothenic acid and of its calcium salt on rats depleted for 17 days on the basal ration is shown in figure 2. The growth response to the daily administration of 100 micrograms of calcium pantothenate or its equivalent of both esters was immediately and approximately the same in each case. Within 3 weeks the quality of the fur of the rats in these groups improved considerably, and discharge from the nose, rusty spots on the fur and "blood-caked" whiskers had disappeared. The rats which received during the same period identical amounts of the test substances autoclaved for 20 minutes at pH 14, failed to gain in weight and did not differ in their appearance from the untreated control animals.

One of these esters (ethyl monoacetyl pantothenate) was tested on a group of chicks maintained on a ration in which pantothenic acid had been destroyed by prolonged heating (3). Supplementation with ethyl monoacetyl pantothenate prevented the occurrence of dermatitis and promoted growth comparable to that obtained with calcium pantothenate.

Discussion. The data presented demonstrate a striking difference in the degree of utilization of both ethyl monoacetyl pantothenate and ethyl pantothenate between Lactobacillus casei and rats or chicks. While rats apparently utilize these esters to the same extent as calcium pantothenate, their utilization by Lactobacillus casei is almost negligible. However, hydrolysis (particularly by autoclaving at pH 7) increases to some extent the activity of both esters on the growth of Lactobacillus casei. Woolley et al. (8) reported that acetylation of pantothenic acid destroys its activity on chicks and more recently Woolley (9) reported that pantothenic acid diphosphate is biologically inactive when assayed by the bacterial method. On the other hand, Grüssner et al. (1) found that ethyl pantothenate had a growth promoting effect on rats maintained on a diet presumably free from pantothenic acid. Apparently, neither of these investigators tested their compounds on both microörganisms and laboratory animals. Furthermore, Mitchell et al. (4) found it necessary to hydrolyze methyl acetyl pantothenate for the determination of its physiological activity on Streptococcus lactis. Our experiments demonstrate that certain esters of

pantothenic acid can be as effective as the calcium salt of pantothenic acid in rats, but are utilized to only a very small extent by Lactobacillus casei. They indicate, therefore, that microbioassays with Lactobacillus casei determine the free pantothenic acid, but may fail to give information concerning the presence of pantothenic acid in the form of esters which can be utilized by animals.

In this respect our findings are analogous to those made on the tetracetate of riboflavin (6) which was found to be inactive in the bacterial test, but active on rats.

SUMMARY

Two esters of pantothenic acid, ethyl monoacetyl pantothenate and ethyl pantothenate have been studied for their biological activity on Lactobacillus casei and on rats and chicks.

1. Both esters were found to be only slightly active in stimulating growth of Lactobacillus casei.

2. Both esters were found to be as effective as equimolecular amounts of calcium pantothenate in promoting growth and in preventing or curing deficiency lesions in rats maintained on a basal diet free from pantothenic acid.

3. The significance of the different utilization of the esters by microörganisms and by animals has been discussed.

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EFFECTS OF ASPHYXIA, ANOXIA AND MYOCARDIAL ISCHEMIA ON THE CORONARY BLOOD FLOW[1, 2]

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Investigations on the perfused isolated heart (2) and the heart-lung preparation (3) show that the coronary flow is increased by hypoxemia, by HCN and by momentary ischemia, but no observations are available regarding the coronary effects of anoxia in the whole animal unless one infers that the increased flow observed during exercise in the unanesthetized animal (4) is due in part to relative anoxia. Furthermore, with the exception of the observations on the fibrillating heart (5), it is not possible to say whether the increased coronary flow in anoxia, asphyxia, etc., is due to effects on the coronary vessels or to extravascular effects resulting from the cardiodynamic alterations produced by the anoxia, or to both (6, 7). In order to investigate critically the effects of asphyxia and anoxia in the whole animal and at the same time to analyze the mechanisms involved, the aortic pressure and the moment to moment inflow into a coronary artery of anesthetized dogs were optically recorded.

PROCEDURE. Twenty dogs, averaging around 15 kgm., were anesthetized subcutaneously with 40 mgm. morphine and sodium barbital (200 mgm./kgm. intravenously). Under artificial respiration, the heart was exposed and suspended in a pericardial cradle. The animal's blood was rendered noncoagulable by intravenous injection of 75 units of heparin and 80 mgm. of Calcomine fast pink[3] per kgm. of body weight. A cannula was inserted into the ascending aorta by way of the subclavian artery, and another directed peripherally, was inserted into the ramus descendens anterior. Following a procedure previously described (8), the coronary flow was optically recorded by conducting the blood from the aortic cannula through an orifice meter and thence into the coronary cannula. Arterial blood pressure was recorded by optical pressure manometers connected laterally to the flow system.

The rate of flow was recorded by a beam of light reflected from a mirror in the optical differential manometer connected with the orifice meter. The flow meter was calibrated several times during every experiment by passing blood through

[1] A preliminary report of this work was presented at the Toronto meeting of the American Physiological Society, This Journal **126**: 554, 1939.

[2] Aided by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

[3] Courtesy of the Calco Chemical Company.

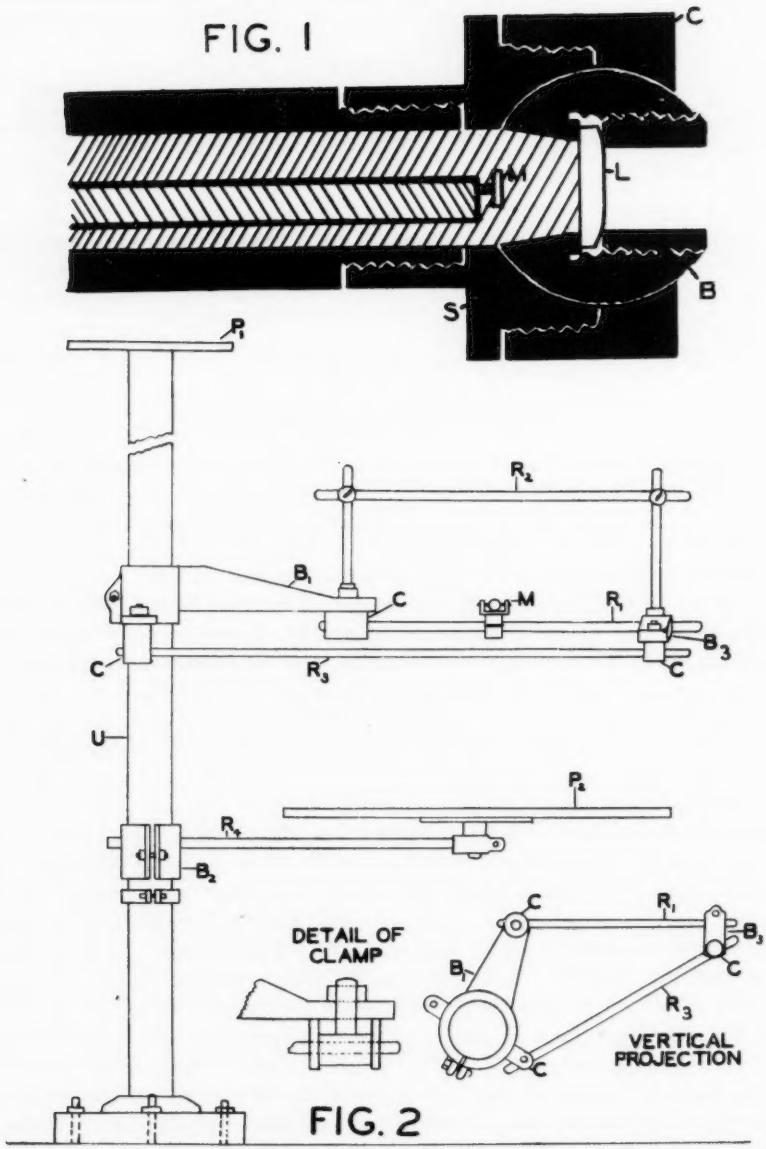

Fig. 1. Ball and socket joint for mounting the window of differential manometers used in liquid systems. See text for description.

Fig. 2. Pedestal stand for mounting optical manometers. U, a vertical steel tube about six and a half feet tall and three and one-half inches in diameter bolted to the concrete floor. B_1, a heavy cast-iron bar; R_1, a one inch rod supporting the manometers, M; a second smaller bar, B_3, and a lighter rod, R_3, united by sleeve clamps, C, form essentially a horizontal triangular mounting. R_2 serves to support the rubber tube connections for filling, flushing and calibrating the manometers. A heavy clamp, B_2, and a one and three-quarters inch steel rod, R_4, support the animal board, P_2. By appropriate adjustment of the various clamps this board can be elevated, rotated and moved to any position under the manometers, or swung to one side free of the manometers to provide an operating table. P_1, a platform for supporting the various fluids used in filling and flushing the manometers and for giving intravenous infusions. Attached to the post but not shown in the diagram are also the mercury manometer for calibrating the optical manometers, an artificial respirator and electrical, pressure and vacuum outlets.

it at known rates of flow while recording the deflection of the differential beam. The deflection of this beam is roughly proportional to the square of the rate of flow through the orifice. The instantaneous rate of flow is determined by comparing the deflection of the beam at the desired instant in the cycle with the calibration scale placed adjacent to the record. The total flow for any given systolic or diastolic interval was determined by integration of the appropriate interval of the recorded curves after redrawing them with a linear ordinate scale. (See reference 9 for details of the integration procedure.) For convenience in comparing these at different heart rates the flow for a single systolic or diastolic interval (in cubic centimeters) was multiplied by the heart rate to give the total systolic or diastolic flow in cubic centimeters/minute. The mean flow, equal to the total cycle flow per minute, is the sum of the total systolic and total diastolic flows per minute.

A number of improvements in the method of recording were made. Since it is rarely possible to mount the mirror of the differential manometer so that it will be parallel to the window, a prism of water is usually present which causes colored fringes and therefore poor definition of the recording beam. In the original model a small angle prism was placed in front of the window to correct for the prismatic effect of the water. In these experiments a differential manometer was used in which the window (see fig. 1, L), mounted in a leak-proof ball, B, and socket joint, C, S, could be rotated until it was parallel to the mirror, M. This arrangement[1] made it possible to obtain much sharper images and to increase the light intensity. The differential manometer and pressure manometers were mounted on a special optical stand[1] (see fig. 2) which provided the rigid mounting necessary for optical recording yet allowed easy access to both the manometers and experimental animal. A description of the parts of the stand is given in the legend accompanying the figure.

Results. Asphyxia was studied in three experiments by shutting off the artificial respiration. The results are illustrated in figure 3 by sections of records taken from a typical experiment. The upper two curves, AP_1, AP_2, are records of aortic pressure; the lower curve, F, is the record of coronary flow. In the control period with a systolic aortic pressure of 93 mm. Hg and a diastolic pressure of 52 mm. Hg, the instantaneous rate of flow at the end of diastole, A, was 25.5 cc./min. During isometric contraction which follows, A, backflow into the aorta was observed, followed by forward flow which reached a peak during the rise of aortic pressure and then dropped to a steady rate of 4 cc./min. After the end of systole, B, the rate of flow increased rapidly during isometric relaxation, reached a peak in mid-diastole, and then slowly declined until the next isometric contraction, A'. The total flow during systole, $A-B$, was 0.017 cc., and that during diastole, $B-A'$, was 0.113 cc. These figures times the heart rate (135) give total systolic and diastolic flows of 2.3 cc./min. and 15.2 cc./min. respectively. The mean cycle flow calculated from the sum of these two was 17.5 cc./min.

[1] A preliminary description of this apparatus was presented at the Toronto meeting of the Am. Physiol. Soc. (10).

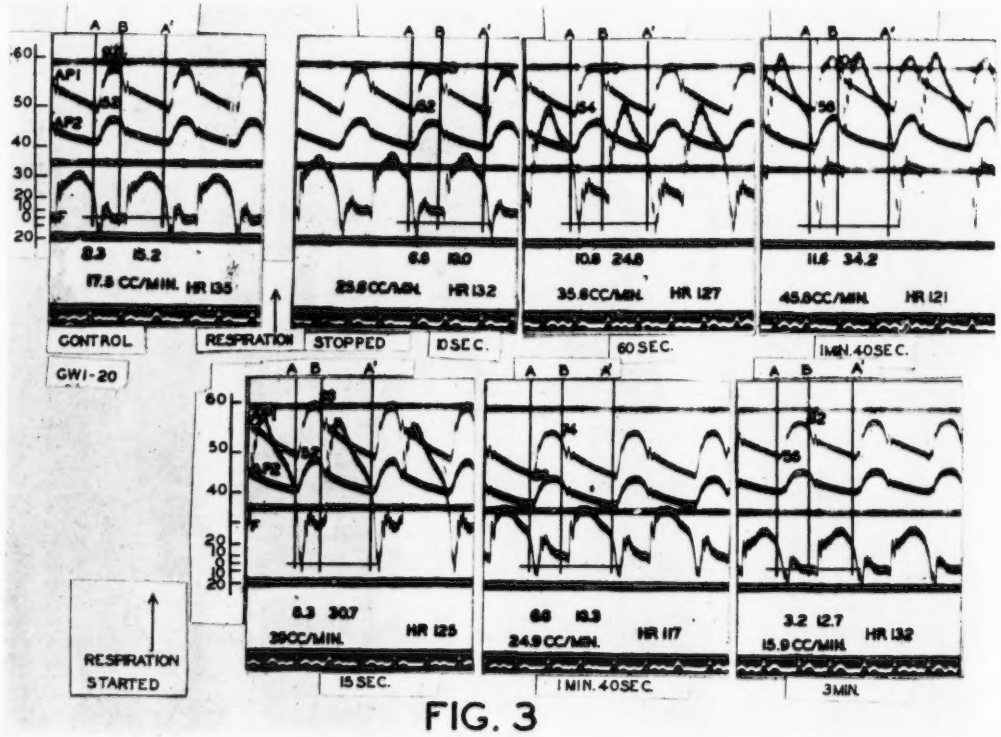

FIG. 3

Fig. 3. Segments of records showing effects of asphyxia on coronary flow and aortic blood pressure. Asphyxia produced by stopping artificial respiration at ↑. AP_1, aortic pressure recorded by a manometer connected laterally to blood stream proximal to meter. The maximum and minimum pressures in mm. Hg are indicated by the figures adjacent to the curves; AP_2, aortic pressure recorded similarly by a manometer connected distal to meter; F, record of coronary flow (see scale at left of curves giving the instantaneous rate of flow in cc./min.—read to top of line). A and A', end of diastole—i.e., beginning of isometric contraction. B, end of systole, i.e., onset of protodiastole. Figures immediately underneath flow curve indicate the total systolic and diastolic flows per minute. The mean cycle flow is the sum of these two. $H.R.$, heart rate.

Within 10 seconds after discontinuing the artificial respiration the flow at the end of diastole, *A* or *A'*, had accelerated to 31 cc./min., and that at the end of systole, *B*, to 17 cc./min.; the total systolic flow had increased to 6.6 cc./min., and the diastolic to 19.0 cc./min., and the mean cycle flow to 25.6 cc./min., despite the absence of any essential change of aortic pressure or heart rate. At the end of 1 minute there was still no significant change in aortic pressure or heart rate, but the instantaneous flows at the end of systole, and at the end of diastole, the total systolic and diastolic flows and the mean flow had increased to 690, 160, 470, 165 and 205 per cent of their respective control values. As asphyxia was continued, the coronary flow increased still further, but at that stage the blood pressure had risen and the heart had slowed.

As shown by the other segments of figure 3, after artificial respiration was restored, blood pressure, heart rate and coronary flow progressively returned to their control values. However, after 1 minute 40 seconds the coronary flow was still significantly increased, whereas blood pressure and heart rate were below their control values.

Anoxia and hypercapnia were produced separately by giving artificial respiration by means of a reciprocating pump supplied with gas mixture stored in Douglas bags. Respiration of a mixture of 10 per cent by volume of oxygen in nitrogen, for periods up to 4 minutes, caused no appreciable changes in either blood pressure or coronary flow. Longer periods were not used because of the difficulty of storing the large volumes of gas needed. In all 11 trials in 5 animals with oxygen percentages of 7 per cent or less, however, essentially the same effects as the ones produced by asphyxia were observed. In a typical experiment, illustrated in figure 4, the instantaneous rate of flow at the end of systole was 10 cc./min. and that at the end of diastole, 41 cc./min.; the total systolic flow was 9.9 cc./min. and diastolic 20.4 cc./min., and the mean cycle flow was 30.3 cc./min. Within 20 seconds after starting respiration of 5 per cent oxygen, the instantaneous rate of flow at the end of systole had increased to 18.5 cc./min. and that at the end of diastole to 57 cc./min.; and the total systolic, diastolic and mean cycle flows were respectively 13.6 cc./min., 25.9 cc./min., and 39.5 cc./min., despite a slightly lowered aortic pressure. After 94 seconds of anoxia the instantaneous rates of flow were increased to 340 per cent of the control value at the end of systole, and to 210 per cent at the end of diastole, and the total systolic and diastolic and mean cycle flows were respectively 230, 215 and 220 per cent of their control rates, with the aortic pressure restored to about the control level. Within 48 seconds after returning to respiration of air, the aortic pressure and rate of flow were restored to the control level. The top curve in this record was obtained with the myograph of Tennant and Wiggers (11). It shows no apparent effect on the contractile effort as a result of the anoxia. In 14 tests on 5 animals, all of which exhibited the typical responses to asphyxia, anoxia or myocardial ischemia described in this paper, CO_2 in air in concentrations of five to eight per cent by volume was administered until slowing of the heart, premature systoles and decline of aortic pressure occurred. No significant increase of coronary flow was observed. Slight decreases of flow at the end of diastole

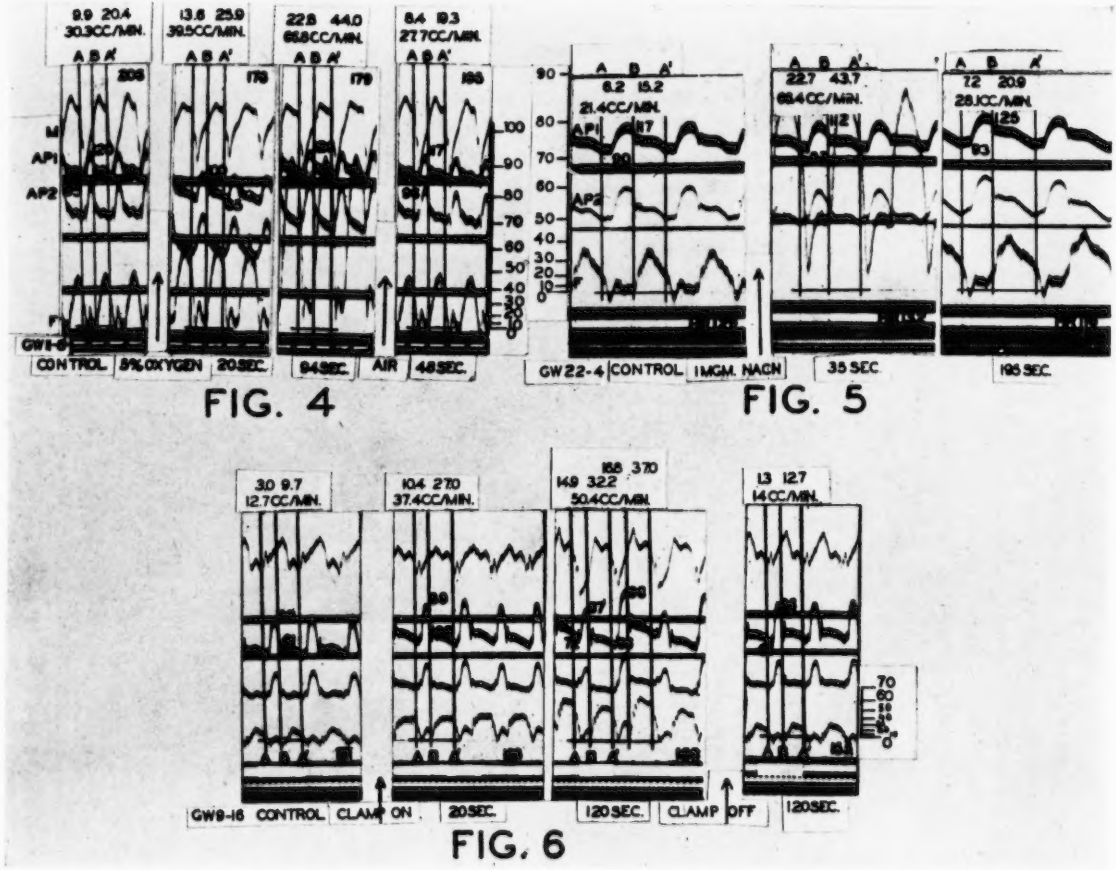

FIG. 4

FIG. 5

FIG. 6

Fig. 4. Effects of artificial respiration with 5 per cent by volume oxygen in air on coronary flow and aortic pressure. See figure 3 for lettering. *M*, record of myocardial contractions, upward movement of the line indicates shortening of the myocardial fibers.

Fig. 5. Effects of temporary ischemia of the coronary bed on the coronary flow. See figure 4 for lettering.

Fig. 6. Effects of an intracoronary injection of 1 mgm. NaCN on the coronary flow and aortic pressure. See figure 3 for lettering.

were often seen but they usually accompanied a decline of aortic diastolic pressure.

Myocardial ischemia. The observations of Gregg and Green (12) indicated that temporary myocardial ischemia produces markedly increased coronary flow. This was investigated more thoroughly in these experiments in order to correlate the effects of generalized asphyxia with local ischemia. The myocardial ischemia was produced by temporarily preventing blood flow into the area of the myocardium vascularized by the ramus descendens anterior. At periodic intervals blood was then allowed to flow into this area through the meter for a few seconds to record the effect of the ischemia. The results in a typical experiment are reproduced in figure 6. Again the effects were comparable to those of generalized asphyxia. The first flow measurement was made after 20 seconds of ischemia and at this time significant increases of flow were recorded. After 120 seconds of ischemia the instantaneous rates of flow at the end of systole and the total systolic flow were increased to over 500 per cent of the control, and the instantaneous flow at the end of diastole, the total diastolic and the mean cycle flows were increased to over 350 per cent of the control values. The myograph, flow and blood pressure curves show that, after two minutes of ischemia, the heart began alternating due to decreased systolic shortening in the ischemic area every other beat (see ref. 17). As shown in table 1, the instantaneous rates of flow at the end of systole and diastole in the strong beats were somewhat greater than in the weak beats when compared to the simultaneously existing aortic pressures. Corresponding observations were made in 28 tests on 15 animals. Only one animal (GW15) showed no change in coronary flow in response to myocardial ischemia. This animal also failed to react to intracoronary injection of the nitrites (13).

One of the most interesting points in our experiments is the brief period of ischemia required to produce an increase in coronary flow. In all 9 trials in 4 animals, a period of ischemia of 3 to 5 seconds sufficed to increase the instantaneous coronary flow at the end of systole to 150 to 250 per cent of the control value, and that at the end of diastole to 130 to 200 per cent of the control value. With ischemia lasting 1 to 2 minutes the rates of flow approached their maximum at about 45 seconds to 1 minute. The maximal flows recorded were around 200 to 400 per cent of the control values for the flow at the end of systole and around 200 to 350 per cent for the flow at the end of diastole. A significant change of aortic pressure was not observed in any of these experiments. With periods of ischemia of 1 to 2 minutes recovery of normal rates of flow usually occurred within 2 to 3 minutes. Since longer periods of ischemia failed to produce any further increase of flow, these rates recorded may represent maximal vasodilatation. Also of interest is the fact that a significant impairment of myocardial contraction, as indicated by the systolic extension recorded by the myograph, did not appear before thirty seconds to one minute of ischemia, whereas by this time coronary flow had approached its maximum. Furthermore, during recovery myocardial contraction became practically normal within one to one and a half minutes, whereas return of the coronary flow to its normal value always required two to three minutes.

Myocardial anoxia. To determine whether the effects of myocardial ischemia like those of generalized asphyxia could be due to anoxia, we made 5 injections of 0.2 to 1.0 mgm. NaCN into the cannulated coronary vessel in 5 animals. The results, essentially the same in all the experiments, are illustrated by the segments of records in figure 5. The maximum effect, observed about 35 seconds after the injection, consisted of an increase of the instantaneous and total systolic flows to over 370 per cent of the control, an increase of the diastolic flows to over 280 per cent, and of the mean cycle flow to around 310 per cent of the control rates, despite the absence of any significant change of aortic pressure or heart rate. Complete recovery occurred in 195 seconds.

Discussion. The most striking fact demonstrated by these records is the marked increase in mean flow which preceded any rise of aortic pressure and must therefore have been due to decreased resistance to flow in the coronary vessels. Since in many instances the percentage increase in the systolic contribution was even greater than in the diastolic one is led to suspect that in addition to relaxation of the vascular walls some mechanical factor affecting the extravascular compression was in operation.

As indicated elsewhere (8, 13, 18), comparison of the experimental instantaneous rate of flow at the end of diastole (A or A^1 in the figs.) with that at a corresponding instant in the control record gives a measure of the change in intramural flow mainly due to intrinsic activity of the vascular walls. Similar comparison of the instantaneous flows at the end of systole (B in the figs.) gives in addition an indication of the alteration in the effective extravascular pressure.

Were it not for the changes of aortic pressure such comparison could be made directly for the flows. In order to allow for the effects of changing aortic pressure we have assumed that over the range of pressure fluctuation observed the flow would vary proportionally with the aortic pressure and have expressed our data as a ratio of flow to pressure-index of flow (13).

The systolic and diastolic indices of flow for the experiments illustrated in this paper are given in table 1. An additional column contains a ratio of the systolic to the diastolic index. It is seen that both indices are increased as a result of asphyxia, anoxia and myocardial ischemia and anoxia. Furthermore, as shown by the last column, the systolic index was in all instances increased relatively more than the diastolic. Of interest in this connection is the fact that the coronary dilator action of the nitrites and especially of the xanthines was usually accompanied by a smaller increase in the systolic index than the diastolic index (13). It seems therefore that the effects of anoxia, asphyxia, etc. are both to cause relaxation of the coronary vessels and also to decrease the effective extravascular compression exerted during systole.

According to Strughold (14) anoxia causes the ventricles to be larger in both systole and diastole. This may have operated mechanically to decrease the effective extravascular compression force, especially during systole, even though the tension per unit of cross section of the myocardium were increased — as it must be with an enlarged and therefore thinner walled chamber if the same systolic aortic pressure is developed (15). The larger systolic ventricular size may be the cause of the greater systolic index of flow during the large beats in

the ischemic heart. This concept is consistent with the effects of increased venous return on coronary flow (12) and on the relation of intramyocardial pressure to aortic pressure observed by Johnson and Di Palma (16). The indices suggest that the coronary vascular lumen continues to increase during the phase of rising aortic pressure.

Whatever the mechanism, the effects of asphyxia, anoxia, and myocardial ischemia and anoxia were essentially similar as regards the magnitude and character of their effects. The absence of any significant effect with hypercapnia

TABLE 1

Tabulation of typical experiments on coronary flow

NUMBER	EXPERIMENT	*SF*	*SP*	$S\frac{F}{P}$	*DF*	*DP*	$D\frac{F}{P}$	$\frac{S\frac{F}{P}}{D\frac{F}{P}}$
GW1-20	Control	4.0	85	0.047	25.5	52	0.49	0.096
	Resp. off 10″	17.0	85	0.20	31.5	52	0.605	0.33
	Resp. off 60″	27.5	90	0.306	40.5	54	0.75	0.408
	Resp. off 1′ 40″	37.0	98	0.378	50	58	0.89	0.425
GW11-8	Control	10	115	0.087	41	95	0.432	0.201
	5% O_2 64″	34	122	0.279	86	101	0.852	0.328
GW9-16	Control	2.5	75	0.033	18.5	61	0.303	0.109
	Ischemia 2′ (small beat)	29	81	0.358	56	66	0.850	0.422
	Ischemia 2′ (large beat)	41.5	91	0.456	63	72	0.875	0.52
GW22-4	Control	12.5	115	0.109	25	90	0.278	0.289
	NaCN (1 mgm.)	50	100	0.50	72	84	0.857	0.583

Number = animal number and experiment number. Experiment = type of experiment performed. *SF* = instantaneous rate of flow at end of systole, i.e., at *B* in figures. *SP* = systolic aortic pressure at *B* in figures, note this figure is slightly lower than the peak pressure indicated in the figures. $S\frac{F}{P}$ = systolic index of flow = systolic instantaneous flow divided by simultaneously existing aortic head of pressure. *DF* = diastolic instantaneous rate of flow—at end of diastole, i.e., at *A* or *A′* in figures. *DP* = diastolic aortic pressure, $D\frac{F}{P}$ = diastolic flow index = diastolic instantaneous rate of flow divided by the simultaneously existing aortic head of pressure.

suggests that, under the conditions of these experiments, the effects of asphyxia were due almost solely to the anoxia produced. The regularity of the reaction to ischemia is so marked that it has become our custom in studying drugs to test the reactivity of the coronary bed by noting whether a significant increase of flow follows a ten second period of ischemia.

The reaction of anoxia serves a useful purpose since it occurs prior to any indication of impairment of myocardial contraction (see figs. 4 and 5). It is not possible to establish from these experiments whether the responses to anoxia are produced by the direct action of the anoxia upon the vascular bed or through reflexes initiating perhaps from the carotid sinus, but the fact that the increased

index of flow can also be produced by local anoxia suggests that local anoxia is at least a mechanism, if not the major mechanism, involved.

SUMMARY

The effects of systemic and myocardial asphyxia and anoxia on the coronary circulation of dogs anesthetized with morphine and sodium barbital have been studied by metering with an optically recording orifice-meter the rate of flow of blood from the aorta into a coronary artery while the aortic head of pressure was measured by an optically recording pressure manometer. Both of these instruments were of adequate frequency to record the details of the contour of the flow and pressure fluctuations occurring within a single heart cycle.

Asphyxia produced by interrupting the artificial respiration, and local ischemia produced by temporarily stopping the blood flow to an area of the myocardium, both caused increased coronary flow even in the absence of any change of aortic pressure. Since the responses to hypercapnia were negligible under the conditions of these experiments, whereas the effects of systemic anoxia—produced by artificial respiration with mixtures of air and nitrogen—and myocardial anoxia—produced by cyanide injections into the coronary artery—were essentially similar, it is concluded that they all depend upon the anoxia produced and probably upon this anoxia being present in the myocardium.

Since the ratio of flow to pressure—index of flow—was increased even more in systole than in diastole, it is concluded that the anoxia causes both a relaxation of the walls of the coronary vessels and also alters the dynamics of the myocardium in such a way that the systolic tension development in the myocardium is less effective in producing the extravascular compressing force normally present during ventricular systole.

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A STUDY OF THALAMO-CORTICAL RELATIONS

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The present study is a topographical analysis of thalamo-cortical relations as revealed by alterations in the electrocorticogram induced by localized thalamic stimulation. The work has been based upon the assumption that the complex spontaneous pattern of the cortex represents an aggregation of various sorts of activity, some of which might have separate anatomical substrates capable of more or less discrete activation. Evidence has already been presented (Morison, Dempsey and Morison, 1941b) for the existence of at least one such system (in that case extrathalamic).

METHODS. Cats under various degrees of nembutal anesthesia (0.6 to 0.9 cc./kgm.) were used. Both cortices were exposed and the stereotactic instrument described by Morison, Dempsey and Morison (1941a) applied to the skull. The stimulating electrodes consisted of two enameled stainless steel wires held in the barrel of a 21-gauge hypodermic needle, the bared tips separated by a distance of about 1 mm. Arrangements for supplying stimulating current were similar to those described before (loc. cit.) except that a resistance of 10,000 ohms was inserted between the center tap of the Wagner balancing potentiometer and ground.

Recording electrodes consisted either of silver wires arranged in pairs with an interpolar distance of 1 mm. or silver-silver chloride-Ringer-agar wicks making a somewhat diffuse contact with the pia (3–6 sq. mm.). When the wicks were used, records were usually taken between each of them and an "indifferent" one placed on the muscles of the neck. When the silver wires were employed, as was usually the case, similar "monopolar" recordings were taken between one of the members of the pair and an "indifferent" point. When responses appeared, changes were made by means of selector switches so that records were taken between the two members of a single pair. This is not the place to discuss the very important theoretical objections which may be advanced against the two methods of recording, but in general it may be said that the data assembled have been analyzed with two major considerations in mind. Absence of a response on a diffuse monopolar lead points to a lack of significant electrical activity in a relatively wide surrounding area, while presence of response recorded with bipolar electrodes indicates activity very near (less than a millimeter) to them. Conversely presence of activity on the monopolar or absence from the bipolar setting is of less significance.

The recording instruments used were a six channel Grass ink writer and a

DuBois mirror oscillograph driven by the output of five push-pull stage condenser coupled amplifiers.

In general the electrodes were placed on the sensorimotor cortex as follows: on the anterior sigmoid gyrus in two positions, one as medially as possible, and the other at the lateral end of the cruciate sulcus; three positions on the posterior sigmoid and coronal gyri, one medially in the position giving the greatest response to sciatic stimulation, a second somewhat more laterally and posteriorly in an area giving responses to the radial nerve, approximately position R 2 of Marshall, Woolsey and Bard (1941) and a third as far laterally as was easily exposible. It was hoped that the lateral electrode position was in the neighborhood of the sensory face representation and in some experiments this was verified by recording potentials here in response to slight mechanical stimulation of the face. It was impractical, however, to make a complete map of the cortex by peripheral stimulation in each preparation and to follow it with a map of the thalamocortical projection. As the experiments progressed, it became clear that the localization of thalamic stimulation was at all events not equal to the task of making sharp distinctions between arm, leg and face areas in the cat.

In several experiments a third set of electrodes was placed in a line 6 to 10 mm. behind those on the posterior sigmoid gyrus so that they made contact with the anterior marginal, posterior coronal, and anterior suprasylvian gyri. Potentials were rarely recorded on any of these electrodes without concomitant activity in those placed on the posterior sigmoid, and in making the thalamic map illustrated below only the latter were specifically considered. In four experiments the frontal pole of the opposite hemisphere was cut away and electrodes placed upon the medial surface of the gyrus proreus.

The usual experimental procedure was as follows: all electrodes were connected to a selector box which allowed any possible combination of two to be fed to each of the six channels of the ink writer or to the amplifier supplying the DuBois instrument. Arrangements were then made by the use of the selector to record from six positions with the monopolar method. The stimulating electrodes were then lowered into the thalamus by 1 mm. steps, the most medial and posterior position being explored first. In most of the experiments four or five sagittal planes and three to five transverse planes separated by 2 mm. were thus explored. Cortical positions giving moderate to large potentials were noted in the protocol for each thalamic point. Relatively small (20 per cent or less of maximal) potentials were in general ignored when the monopolar recording system was employed. In cases of doubt, the responses were analyzed by means of bipolar records, which were found to have considerably more localizing value than had the monopolar method. When more accurate information in regard to latency and wave form was desired, photographic records were taken.

At the end of the experiments the brain was removed and fixed in formalin. In the majority of experiments sections were cut free hand and stimulation points identified by macroscopic inspection with reference to the coördinates of the stereotactic instrument. In others the tissue was imbedded in celloidin in the usual way, cut at 30μ, and alternate 5th sections stained with the Nissl and

Pal-Weigert methods. The active thalamic points identified by both these methods were plotted on enlarged tracings of the thalamus and its principal nuclei taken from the plates shown in the paper by Ranson and Ingram (1932). The nomenclature of the thalamic nuclei cited in this paper is therefore that of the same authors. For the final figures the number of plates was reduced by incorporating the points on two successive plates in one, a procedure which seemed justified, since the stimulus employed spread over at least a millimeter.

RESULTS. Several types of cortical responses may be elicited by thalamic stimulation, some of which have been mentioned elsewhere (Morison, Dempsey and Morison, 1941a). As might be expected from the intricacy of the fiber connections to, from, and within the thalamus, cortical response to stimulation within the thalamus may be quite complex and variable. Two general types

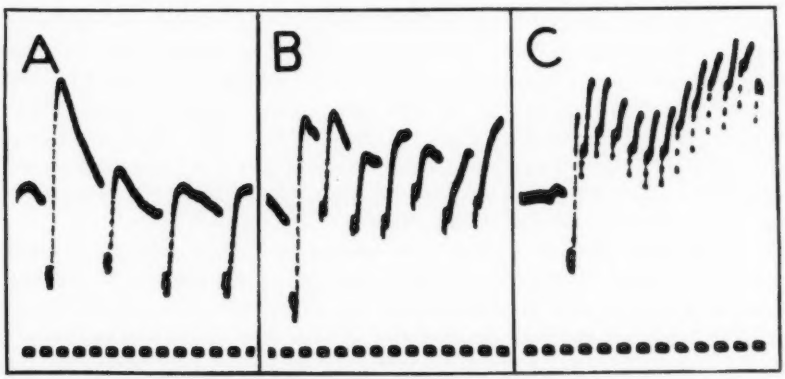

Fig. 1. The primary response as recorded from a monopolar electrode on lateral part of g. coronalis at different frequencies of stimulation in the region of medial tip of pars externa. A, 30 per sec. B, 60 per sec. C, 120 per sec. Note the relatively rapid disappearance of the negative phase. In this and the ensuing figures time is in 10 msec. intervals.

with widely different properties may, however, be easily separated out and some of their variants identified. These may be schematized as follows.

A. *The "Primary" Response.* 1. *Description of response.* Stimulation of the lateral thalamic mass gave rise to well-localized responses in the sensorimotor cortex. In the region of greatest activity these consisted of an initial deflection which was positive to the putative "active" lead. This was ordinarily followed by a negative deflection of varying degree and constancy, frequently recordable over a wider area than was the initial positive activity. Both phases of the response sometimes increased in magnitude in response to succeeding stimuli at moderate frequencies (5 to 30 per sec.). At higher frequencies marked declines in both phases of the potentials were recorded, the negative being the most labile (fig. 1A). Frequencies of 60 per second usually reduced the responses to a fifth or less of their initial value in a few seconds. A few preparations gave appreciable responses for a considerable length of time at frequencies as high

as 120 per second (fig. 1). No special study of this point was made, but it appeared that the conditions favorable for higher frequency of response were: light anesthesia, and stimulation distal to the thalamic projection nuclei, i.e., the thalamo-cortical radiations. Especially striking ability to follow higher frequencies was exhibited by responses recorded from the anterior sigmoid gyrus to stimulation in N. ventralis pars arcuata. In several instances the deflections recorded in the latter part of the series bore a striking resemblance to a series of sine waves, an effect presumably due to temporal dispersion. No lower limit may be assigned for the latency of the phenomenon as the apparatus at hand was not suitable for accurate determinations, but gross latencies as long as 3 to 4 msec. for the positive and up to 8 msec. for the negative deflections have been encountered. In general these times were shorter, the more rostral was the situation of the stimulation points.

Aside from the fact that the negativity tended to be marked over wider areas than was the initial positive deflection, no clear separation has been made between these two phenomena on the basis of thalamic topography. Other evidence that the two phases of the somewhat similar callosal response of Curtis are separable is, however, available (Curtis, 1940). The marked differences in the ability to follow frequency of the two elements (cf. fig. 1) suggest that the negativity is due to cortical elements separated by at least one synapse from those responsible for the initial deflection.

2. *Localization of these responses in sensory and motor regions.* Marshall, Woolsey and Bard (1941) have roughly delimited the cortical area responsive to tactile stimulation as extending from the medial surface of the hemisphere caudal to the cruciate sulcus up over the postcruciate region and including the anterior suprasylvian and ectosylvian gyri. Data derived from threshold stimulation of the sciatic and radial nerves in the present experiments coincide closely with their findings.

Primary potentials occurred in these sensory areas when the thalamic and midbrain regions closely corresponding to the position of the medial lemniscus and of N. ventralis pars externa were stimulated (fig. 2). Stimulation of other regions failed to produce the responses in the sensory cortex.

One qualification of the foregoing generalization is necessary. Stimulation of pars arcuata sometimes produced significant activity near the electrode on the cortical projection of the sciatic nerve. This area occupies what amounts to a narrow peninsula of granular cortex extending into regions which histologically and physiologically are "motor" in type. Various considerations render it very likely that the potentials recorded here in response to activation of pars arcuata arise not in sensory areas but in the surrounding "agranular" regions. 1. The monopolar method increased the potentials recorded, while bipolar records decreased them in comparison with the responses set up by sciatic or pars externa stimulation (figs. 3 and 4). 2. Stimulation of the latter structures almost never produced potentials anterior to the cruciate sulcus when producing their effects in the leg area, whereas activation of pars arcuata always did. 3. The potential produced by pars externa "blocked" with sciatic stimu-

lation (see Dempsey and Morison, 1942, fig. 3), whereas that from pars arcuata did not (fig. 3). 4. Careful removal of adjoining motor areas from the leg

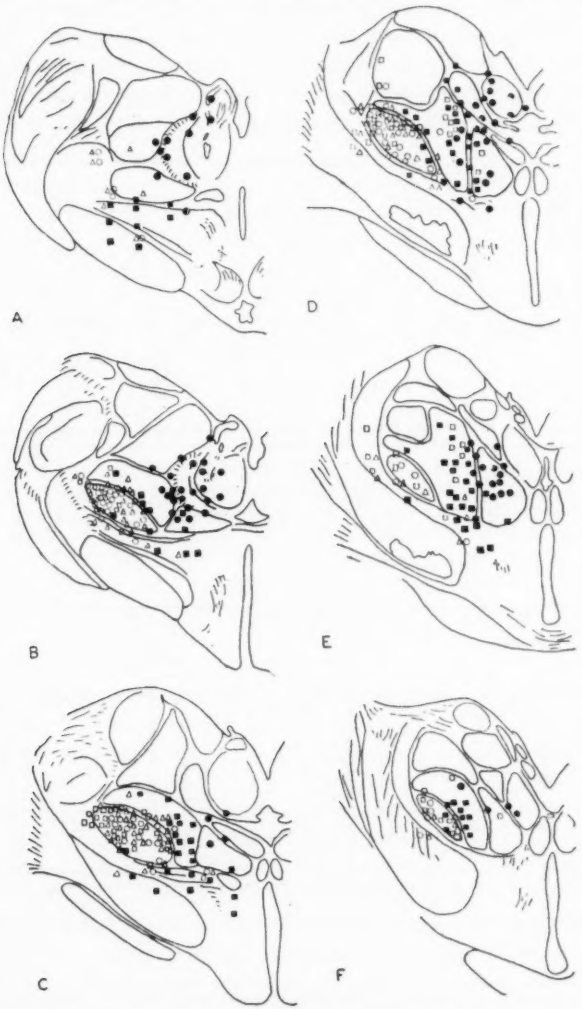

Fig. 2. Sections of the diencephalon taken from Ranson and Ingram (1932) with thalamic points yielding cortical responses plotted upon them as follows: Primary responses: in anterior sigmoid gyri, solid squares; in sciatic cortical projection, hollow squares; in radial projection, hollow circles; laterally on coronal gyrus, hollow triangles. The "recruiting response" (see text) solid circles.

sensory area (one experiment) abolished the activity produced by stimulation of pars arcuata while leaving the sciatic response unaffected.

These considerations make it almost certain that the primary projection of pars externa is discrete from that of pars arcuata, the former being the sensory and the latter the motor, and probably the so-called "premotor" regions, although the latter differentiation is not easily made in the cat. Nevertheless, in making the diagram (fig. 2) all thalamic points which gave significant potentials in the region of the electrode placed over the cortical sensory projection of the sciatic nerve have been included unless proven spurious in the course of

Fig. 3. Record taken from a monopolar lead on the sciatic cortical projection. A, response to sciatic stimulation. B, response to stimulation of pars arcuata at 20 per sec. C, D and E, interaction of the two stimuli at various intervals. Note absence of block; if anything the negative phases tend to sum.

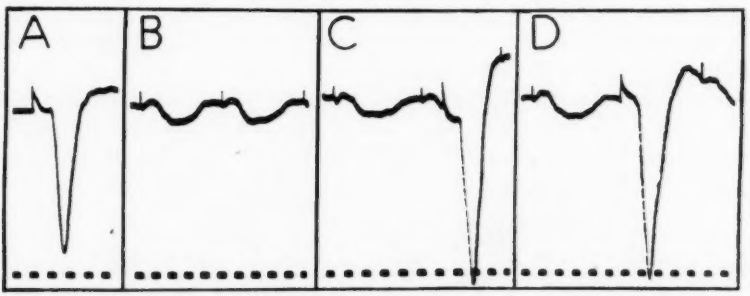

Fig. 4. Bipolar records of the responses in figure 3. Note the extreme reduction of the pars arcuata response.

the same experiment. Similar difficulties were not so severe when dealing with the cortical representation of the radial nerve, except that points near the medial tip of externa frequently yielded potentials on both motor and sensory regions. Since stimulation more medially gave responses purely in "motor" regions, while those more laterally gave purely "sensory" effects, this confusion was not serious (see fig. 2, especially C and D).

Localization of arm and leg areas within pars externa cannot be definitely established on the basis of these experiments. In the course of any single experi-

ment, shifting the stimulating electrodes by as little as 1 mm. in the thalamus caused a change in pattern of the cortical response. In general the more medial the thalamic stimulation the more lateral was the major cortical activity. When all the points were plotted together, however, the discreteness of the thalamic points tended to disappear (fig. 2). Undoubtedly afferents to the lateral part of the nucleus traverse more medial regions and medial radiations pass laterally to the capsule so that activation cannot be very discrete. Nevertheless, the most active region for producing responses in the cortical projection of the sciatic seemed to lie quite definitely in a restricted region at the ventral tip of externa underneath the anterior part of the lateral geniculate body (cf. the open squares in fig. 2, C and D). Points most active for the radial nerve projection on the coronal gyrus lay in general more medially in the thalamus.

Stimulation of the posterior part of pars arcuata (fig. 2, B and C), when productive of a recordable response under our experimental conditions, usually gave effects on the anterior sigmoid electrodes, and in many cases most markedly so in lateral positions. Whether this represented activation of the face sensory area said to extend to the ventral tip of coronal sulcus (Waller, 1940), or a motor area lying nearer the electrode, cannot be determined from these experiments as the point was not sufficiently investigated. In the latter and more likely case, stimulation of fibers of the brachium conjunctivum would presumably be responsible, since more ventral stimulation near this region in pars arcuata were shown to have such effects (fig. 2).

3. *Primary responses in other regions.* No detailed examination of primary responses in regions other than sensory and motor cortex were made, but incidental observations which demonstrate the generality of the phenomenon may be mentioned. Stimulation in the region of the geniculate bodies gave rise to responses in various parts of the auditory and visual cortices with a discreteness which suggests that the method would provide a tool for those interested in geniculocortical relationships fully as accurate as the methods heretofore employed.

More interesting perhaps is the fact that similar potentials have been recorded in cortical regions referred to as "association areas." These effects, in the cat at least, appear to be rarer or at least more difficult to obtain than are the responses of primary projection areas. Nevertheless, the gyrus proreus frequently exhibited primary responses when the electrodes were in or near N. medialis dorsalis. Owing to obvious technical difficulties it was not always easy to be sure that the records were not due to electrical spread from motor regions. A further difficulty lay in the fact that the records were necessarily complicated by the later "recruiting" response (see below). A sufficient number of cases exists in which the stimulating electrode was situated as far as possible from pars arcuata and the recorded potential though visible over a part of the anterior sigmoid increased markedly as the electrode approached, to allow one to assert that the primary response recorded was indeed due to activation of the association area.

In three out of four experiments in which specific search was made, localized

responses were also recorded in the middle suprasylvian gyrus when the stimulated points were in the region of lateralis posterior and pulvinar, but not elsewhere in the thalamus.

B. *The "Recruiting Response."* 1. *General description.* In contrast to the well-localized type of "primary" responses described above, an effect of an entirely different character was produced by stimulation of a rather diffuse area in the region of the internal medullary lamina (fig. 2). A detailed description of its properties and evidence that it has much in common with one feature of the spontaneous rhythm observed in nembutalized cats are given elsewhere (Dempsey and Morison, 1942a and b).

For the purposes of the present report it is sufficient to say that it rarely appears significantly in response to a single shock, but recruits rapidly at a critical frequency of from 5 to 15 per sec. This property has given rise to the

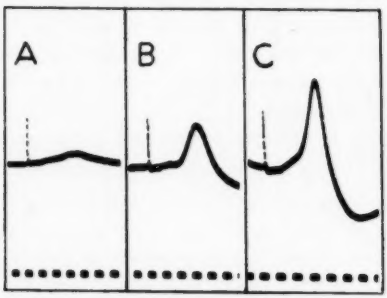

Fig. 5

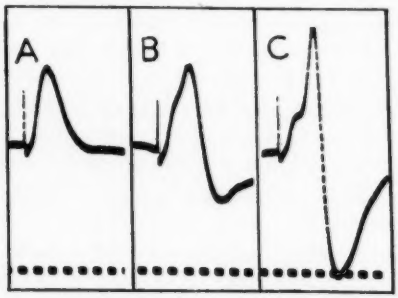

Fig. 6

Fig. 5. Cortical responses of anterior sigmoid gyrus (monopolar recording) to stimulation in region of solid circles as shown in figure 2B. A, B and C, successive stimuli at a frequency of 8 per sec. Note recruitment and long latency.

Fig. 6. Same as figure 5 except stimulus more lateral and anterior as in figure 2E. Note interpolation of a primary response.

tentative appellation of "recruiting response." The latency is very long (20 to 35 msec.) and does not change with repetitive stimulation. The response alternates in height and disappears rapidly at frequencies in excess of 15 per sec. Figure 5 illustrates some of these features. The recruiting response has been produced routinely when the stimulating electrodes were in the neighborhood of the internal medullary lamina and associated structures at levels B, C and D in figure 2 (solid circles). (The relative scarcity of points in fig. 2C is apparently not significant.)

Stimulation further posteriorly has been more capricious. Some preparations gave effects as far back as the posterior commissure, but these were rarely so dependable or so intense as those obtained from the more rostral points. Anterior to level D, especially in lateral positions, complexities frequently occurred in the responses as recorded near motor areas, an effect due to the appearance of "primaries" from coincident stimulation of the ventral nucleus (see fig. 6).

In several preparations excitable areas for the recruiting response were traced

through the anterior pole of the thalamus, beyond the levels illustrated in figure 2 and laterally into the internal capsule. Aside from the frequent mixture with other types of response, no change in the recruiting responses was noted.

2. *Cortical distribution of the recruiting response.* During the course of the preceding experiments upon which the thalamic map was based, it became obvious that the recruiting response was more widely diffused over the cortex than were the so-called "primary" responses. Six additional experiments were carried out in which the stimulating needle was introduced into an active spot at about the level of figure 2 C or D and a map of cortical activity made with a pair of bipolar electrodes recording successively from positions separated by 2 to 4 mm. Repeated observations with slight displacement of the electrodes were made at critical areas where abrupt changes occurred, especially in the small region of large response in the posterior suprasylvian gyrus illustrated in figure 7.

The results were then evaluated largely on the basis of intensity of recorded response, but other characteristics such as dependability, time of recruitment

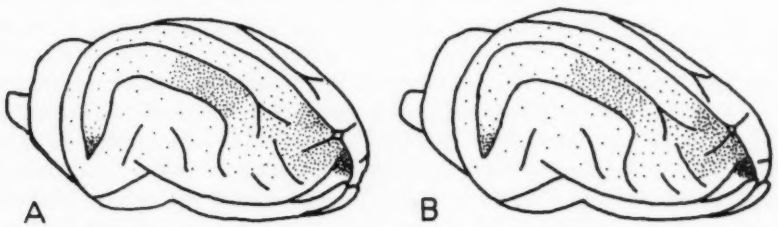

Fig. 7. Cortical map of distribution of recruiting response, A, and of spontaneous 8 to 12 per sec. rhythm, B; for method see text.

and rapidity of onset of alternation were also considered. The cortical areas were graded on a 5-point scale and the results plotted in figure 7A where the intensity of stippling varies directly with the amount of "recruiting" activity produced. The number of experiments is not large nor the method impeccable, but the data were so consistent with themselves and with various other considerations which appear later that they seem worth submitting.

It will be seen from the figure that all parts of the readily exposable cortex participated in the recruiting response, but that the most striking results were recorded on the gyrus proreus, the middle suprasylvian, and a small triangular area at the lower margin of the posterior suprasylvian gyrus.

During the course of each of the experiments a similar map of the spontaneous bursts of 8 to 12 per sec. activity, a prominent feature of the records of nembutalized cats, was made. The results are plotted in figure 7B and were found to be identical within the limits of the method with those obtained from plots of the recruiting response (fig. 7A).

Discussion. Attention has already been drawn (Morison, Dempsey and Morison, 1941b) to one item of the electrocorticogram which depends upon subcortical structures other than the thalamus. It should be emphasized at

the outset of the discussion that the present experiments have been designed with a view to "dissecting" in so far as possible the electrical activity of the cortex on the basis of its relations with the thalamus. Emphasis has been placed upon separating the component parts from one another and describing the conditions under which this separation may be effected.

Emphasis on the separability of the phenomena does not necessarily, however, deny the possibility of interaction. Indeed, it is hoped that, once adequate control of the discrete phenomena is established, further work towards knowledge of their integration may be facilitated.

The experiments in section A appear to demonstrate that the cortical areas designated as sensory on other grounds, histological (cf. Kappers, Huber and Crosby, 1936, for references), and physiological (Marshall, Woolsey and Bard, 1941) may be shown to exhibit electrical activity of a specific sort when other cortical areas are silent. Activity, at least in regard to arm and leg areas, may be set up by localized stimulation in the region of N. ventralis pars externa in cats under moderate to deep nembutal anesthesia. This is thoroughly consistent with the knowledge that the medial lemniscus ends in this nucleus (Ranson and Ingram, 1932) which in turn projects to the sensory cortex (cf. Waller, 1940, and others).

Similarly stimulation along the course of the fibers of the brachium conjunctivum and its relay nuclei more anteriorly yielded effects anterior to the sensory cortex in motor or "premotor" regions (fig. 2). Whether or not the anterior active region is to be thought of as an anterior extension of pars arcuata (Ingram, Hannett and Ranson, 1932) or a posterior extension of ventralis anterior is a question better left to others (cf. Waller, 1940, for references). We have adopted the former nomenclature since the figures of Ranson and Ingram were used as a basis for figure 2 in this paper in order to form a ready comparison with the course of the brachium conjunctivum in their illustrations. As to the projection of the posterior part of pars arcuata and especially its lateral extension dorsal to externa little may be said on the basis of these experiments (see fig. 2). Presumably it represents cortical face areas insufficiently explored in the present experiments.

As is well known, the experiments of Dusser de Barenne and others (cf. Dusser de Barenne and McCulloch for references, 1938) have established functional boundaries between face, arm and leg areas, a distinction in which the present experiments have been less successful for reasons connected with the electrical method of stimulation (cf. section A 2). On the other hand, separation of motor from sensory systems is clearer with the electrical than with the strychnine method. The readiest explanation for the more widespread effects of strychnine is found in the assumption that the maximal stimulation of nuclear structures by the drug opens circuits between motor and sensory areas not activated by localized electrical currents. It is obvious on other grounds that such connections must exist since it is a commonplace of general experience that sensory phenomena modify motor activity and vice versa. Four possibilities for such interaction may be mentioned: 1, the thalamic nuclei may send primary projection fibers to more than one cortical area. This possibility is unlikely on the

basis of anatomical evidence, principally the method of retrograde degeneration; 2, projection fibers of another sort (cf. fig. 63, Lorente de Nó in Fulton's *Physiology*, p. 302, and personal communication) may be sent to adjacent cortical areas; 3, intrathalamic fibers may connect the various relay nuclei; 4, cortico-cortical fibers may be responsible. Evidence that the last of these possibilities plays a significant rôle is found in the many experiments of Dusser de Barenne, McCulloch and others who have demonstrated widespread corticocortical connections between the primary sensory projection centers and adjacent areas ordinarily defined as motor or associative.

The limited experiments on other thalamo-cortical projection systems add physiological evidence to the conclusions of many anatomists (cf. Walker, 1938, and Gerebetzoff, 1937, for references) that each cortical area has specific connections from appropriate thalamic nuclei. They also serve to generalize the "primary" cortical response as applying not only to the sensory projection areas, but to secondary "association" regions as well.

The "recruiting" response is much more difficult to correlate with available anatomical and physiological information. Its widespread occurrence in response to localized stimuli suggests three anatomical possibilities: 1, that the thalamic area stimulated radiates to a localized cortical area whence activity spreads throughout the cortex; 2, that the active thalamic area connects directly to the other thalamocortical projection systems described above and throws them into the special form of activity represented by the recruiting response; 3, that there is a separate neuronal system involving medial thalamic areas which is responsible for the effects. Reasons are advanced in accompanying papers (Dempsey and Morison, 1942a and b) for believing that the first two possibilities are both unlikely. The very long latency and striking temporal summation or recruitment which the response exhibits suggest a diffuse multineuronal system, which can be driven or at least synchronized from a relatively small thalamic region. The similarity in distribution (section B, fig. 7) of the intensity of this response to that of one of the characteristic elements of the spontaneous electrocorticogram suggests that the anatomical substrate of the two phenomena is similar, a conclusion strongly reinforced by other evidence (Dempsey and Morison, 1942a). Particularly interesting is the apparent concentration of effects in the gyrus proreus, middle suprasylvian and a small area in the occipital region, the first two of which at least are well recognized association areas. When one examines the anatomical possibilities available, a suggestion of Lorente de Nó concerning the non-specific thalamocortical fibers (loc. cit.) is the most helpful. His conception (personal communication) is that various thalamic cell groups not ordinarily thought of as projection nuclei send fibers to the cortex which make more diffuse connections than do the specific projection fibers, and may be especially rich in association areas. In his opinion some of these nuclear structures might well be activated in the medial and intralaminar regions, stimulation of which produces the response (fig. 2). It may be pointed out that N. centralis lateralis and the centre median, much of which is included in the active area, have not been found to have specific cortical projections. On the other hand, parts of both have frequently been reported to degenerate in a

variety of cortical lesions, and total degeneration in animals entirely deprived of neocortex has been observed (cf. Waller, 1940, for references). These observations would fit with the diffuse sort of connections necessary for the present purposes.

SUMMARY

1. In cats under moderate or deep nembutal anesthesia thalamic stimulation gave rise after a short latency to a localized response consisting of an initial positive deflection followed by varying amounts of negativity which followed frequencies up to 120 per sec. (fig. 1).

2. Stimulation of the medial lemniscus, its relay nucleus or the radiations therefrom produced major effects in the sensory cortex (fig. 2).

3. Stimulation of the brachium conjunctivum, its relay nucleus or radiations produced major effects in motor or "premotor" areas (fig. 2).

4. Other experiments are noted which suggest that similar primary responses may be produced in any part of the cortex receiving thalamic fibers by stimulation of the appropriate nuclear regions.

5. An entirely different sort of response with special properties (Dempsey and Morison, 1942a) was produced by stimulation in the neighborhood of the internal medullary lamina (figs. 2 and 5).

6. Stimulation anywhere in the active thalamic area produced the phenomenon in all the cortex explored, but the intensity of the response varied in different areas (fig. 7A), and its distribution coincided with the intensity of spontaneous activity (fig. 7B).

7. These observations are discussed and found to support the idea that there exist between thalamus and cortex at least two systems, with very different physiological properties: *a*, the well-known specific projection system with a more or less point to point arrangement; *b*, a secondary "non-specific" system with diffuse connections.

8. The relation of the latter to one element of the spontaneous cortical rhythm is suggested.

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THE PRODUCTION OF RHYTHMICALLY RECURRENT CORTICAL POTENTIALS AFTER LOCALIZED THALAMIC STIMULATION

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During the course of experiments designed to determine the cortical representation of localized thalamic regions, it was observed that stimulation at a frequency of 5 to 15 per second was followed by a recruiting cortical response when the stimulating electrodes were placed in the region of the midline nuclei (Morison and Dempsey, 1942). Moreover, single stimuli delivered to this area frequently produced a train of cortical potentials lasting a few seconds. These trains of spikes were strikingly like the recurrent spontaneous bursts which are seen in the electrocorticograms of animals anesthetized with barbiturates (Derbyshire, Rempel, Forbes and Lambert, 1936). In view of this similarity to a component of the normal spontaneous activity, it was thought advisable to study the effects of stimulation of the medial thalamic nuclei in more detail. The following sections deal with the results of such a study.

MATERIAL AND METHODS. Cats were used. The methods employed have been discussed elsewhere (Morison and Dempsey, 1942) and need not be repeated.

RESULTS. A. *Description of recruiting response.* In cats under relatively deep nembutal anesthesia, in which spontaneous bursts of 8 to 12 per sec. cortical potentials were relatively rare, a slow series of electrical stimuli applied to the dorsomedial regions of the thalamus was followed by recruiting potentials which were widespread in the cortex. The first shock of a series usually produced no response, but following the second or third a cortical potential developed which on successive stimulation increased rapidly in magnitude until a maximum was reached after the fifth or sixth shock. Figure 1 illustrates this recruitment response in an experiment in which the spontaneous 8 to 12 per sec. bursts had been almost abolished by deep nembutal anesthesia. These potentials bore a remarkable superficial resemblance to the spontaneously generated bursts of 8 to 12 per sec. activity (fig. 2).

The recruited potential usually was monophasic and surface negative. After recruitment to its greatest height, however, it sometimes became diphasic with a positive component following the initial negativity. Diphasicity was more frequently encountered in lightly anesthetized preparations. The electrical polarity was not, however, completely constant. Recruited potentials of opposite signs were sometimes recorded from different cortical regions.

The latency of the recruited response was long, varying in different experiments between 20 and 35 msec. In any single experiment the latency was less

variable than is indicated by this range. The duration of the potential usually was 30 to 50 msec., but if the potential were diphasic, longer durations usually were observed.

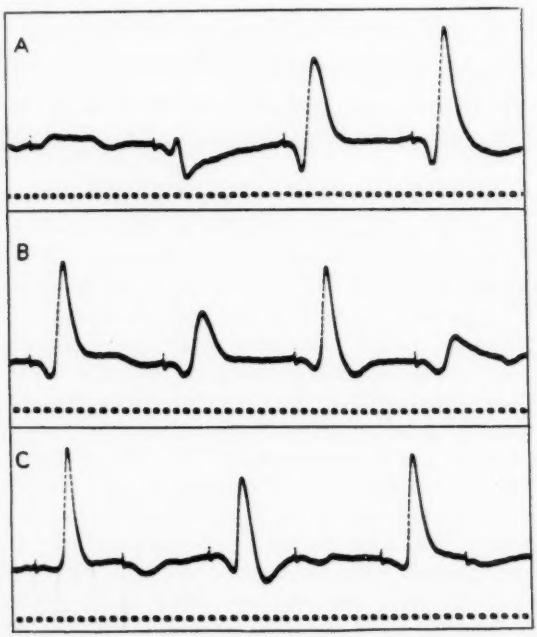

Fig. 1. Monopolar recording, anterior sigmoid gyrus. *A*, recruited potentials developed after 8 per sec. stimulation. *B*, five shocks after the end of record *A*. Alternation has begun, although the largest responses are full-sized. *C*, after 12 per sec. stimulation, alternation of the response is complete. Time intervals in this and succeeding records are 10 msec. unless otherwise noted.

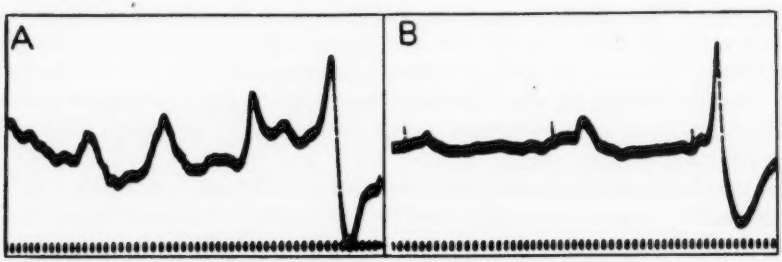

Fig. 2. Bipolar recording, anterior sigmoid gyrus. Similarity of spontaneous and recruited potentials. *A*, beginning of spontaneous burst. *B*, responses to first three shocks of recruiting stimulus.

The voltage of the recruited potential was always large. In several experiments potentials of more than a millivolt were recorded. Careful attention was

not given in this study to absolute voltage, since it has been our experience that the magnitude of cortical potentials is markedly altered by the position, contact and orientation of the recording electrodes. These factors are not easily subject to complete control during the course of any single experiment, and it is therefore considered advisable to specify only the general order of magnitude of the potential.

The wave form was not always as simple as in the responses illustrated in figures 1 and 2. Frequently responses were recorded which showed earlier deflections. For reasons advanced elsewhere however (Morison and Dempsey, loc. cit.), it is suggested that the short latency responses were produced by incidental stimulation of other thalamic structures.

B. *Regions from which recruitment response was recorded.* Stimulation of the dorsomedial thalamic regions led to the appearance of the recruited potentials in essentially all regions of the homolateral cortex. The responses were especially large and simple in form in the gyrus proreus, the anterior sigmoid gyrus and in the middle and posterior suprasylvian gyri. The effects also were present, although smaller and more complex, in the leg, arm and face sensory divisions of the posterior sigmoid gyrus. The visual and auditory cortex produced very poor recruited potentials. A diagrammatic representation of the magnitude of the responses in various cortical regions is presented by Morison and Dempsey (1942, fig. 6).

C. *Regions from which recruited response was induced.* Recruited potentials have been observed whenever stimulating electrodes were placed in the medial thalamic nuclei near the internal medullary lamina at all levels from the posterior commissure to the anterior tubercle of the thalamus, and also when stimuli were applied to the internal capsule. The localization of the regions from which the response may be induced has been discussed elsewhere (Morison and Dempsey, 1942). It is, however, pertinent to remark here that the latency of the response was essentially unchanged whether the stimuli were applied far back in the thalamus or far forward in the internal capsule. Moreover, responses were recorded in all regions of the cortex after stimulation of either posterior or anterior thalamic regions.

D. *Effects of frequency of stimulation.* In cats under fairly deep anesthesia no response was produced by single shocks or by a series at a frequency slower than about 2 per sec. Slightly faster frequencies led to the rapid recruitment described in section A. This recruitment was most rapid when the stimulus frequency was 5 to 10 per sec.

At these frequencies the effect followed the stimulus accurately for 2 or 3 seconds and then declined, occasionally to zero. If the stimulus were continued at the same frequency, a few seconds later recruitment again occurred. When the stimulation was continued for long periods, the periodic recruitment and de-recruitment occurred at longer and longer intervals until finally, after several minutes, it failed completely.

Slightly faster stimulus frequencies (10 to 15 per sec.) usually produced a few responses, after which alternation began. The responses during the period of alternation were full sized. Decline of the potentials was more severe and

occurred somewhat sooner than when a slower stimulus frequency was employed. Moreover, a longer time elapsed before a second period of recruitment occurred.

Still faster frequencies (20 to 120 per sec.) caused a complete disappearance of the response. At 20 per sec. a few responses occasionally were observed, but even these underwent severe alternation from the start.

In more lightly anesthetized cats, medial thalamic stimulation produced bursts of activity which appeared identical to the spontaneous 8 to 12 per sec. bursts which characterize the electrocorticogram after nembutal anesthesia. A single shock frequently caused no immediate response, but after 100 to 150 msec. a train of cortical potentials developed at a frequency of 8 to 12 per sec. This induced burst usually lasted two or three seconds. When a second shock was delivered at the end of the induced burst, a second train of potentials was developed which was identical with the first except that it usually did not last so long. When this procedure was repeatedly carried out, a waning of the effect

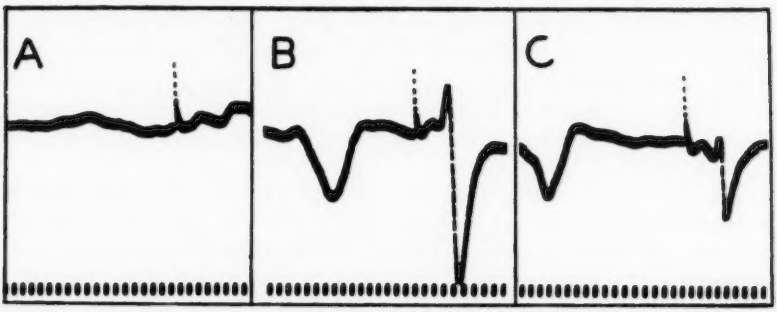

Fig. 3. Bipolar recording, anterior sigmoid gyrus. Variation in recruited response to single shock. See text for detail. *A*, during quiet period between spontaneous bursts. *B*, during beginning of burst. *C*, toward end of burst.

occurred until the burst failed to appear after the fourth or fifth shock. It could be induced at this time, however, if two or three successive shocks were applied at a frequency of 3 to 5 per sec.

In contradistinction to the situation in more deeply anesthetized preparations, a single shock sometimes produced a response in the animals in which spontaneous bursts were common. Moreover, the phase of the periodic spontaneous bursts at which the shock was delivered seemed to determine whether or not a response developed. Fairly large potentials were developed when the shock was delivered just preceding or during the beginning of a spontaneous burst. Toward the end of the burst, the potential generated was smaller. During the silent period following a burst, the responses were either very small or not detectable. Figure 3 shows the responses to three single shocks delivered at different phases of spontaneous activity.

A series of stimuli delivered at a slow frequency in lightly anesthetized animals usually led to the generation of cortical potentials which bore no constant tem-

poral relationship to the stimuli. This was particularly true if the stimulus frequency was slightly slower than the frequency of the burst induced by single shocks. Stimulus frequencies slightly faster than the burst frequency, however, were followed by recruiting responses whose latencies were constant.

When a continuous series of stimuli at a frequency of 5 to 15 per sec. was delivered to lightly anesthetized animals, a periodic waxing and waning of the recruited responses occurred, replacing the spontaneous activity (fig. 4). The interval between these periods of recruitment was short at first, but became

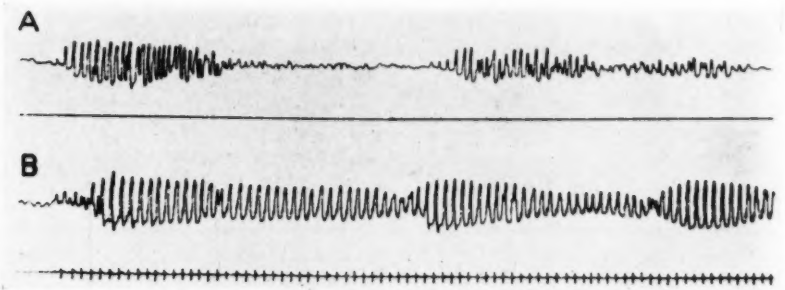

Fig. 4. Bipolar recording, middle suprasylvian gyrus. *A*, spontaneous 8 to 12 per sec. bursts. *B*, continuous stimulus to intralaminar thalamus at frequency shown by signal. Note the waxing and waning of the recruited response. Paper speed, 7.5 mm. per sec.

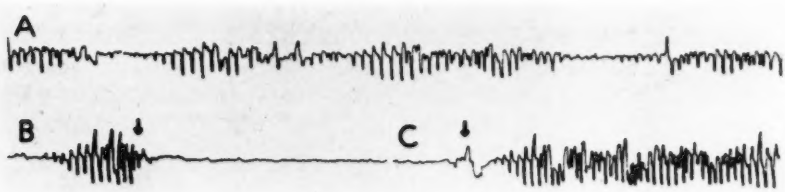

Fig. 5. Bipolar recording, anterior sigmoid gyrus. Abolition of recruited and spontaneous potentials during high frequency stimulation. *A*, spontaneous bursts. *B*, beginning of 60 per sec. stimulus to intralaminar thalamus indicated at arrow. Between *B* and *C* one minute of record has been removed. *C*, end of 60 per sec. stimulation indicated at arrow. Note the prompt return of spontaneous activity. Paper speed, 7.5 mm. per sec.

longer and longer as the length of stimulation was increased. After cessation of stimulation, spontaneous bursts reappeared immediately.

At faster stimulus frequencies (20 to 120 per sec.) the recruited potentials failed to appear in the cortex, and, in addition, the spontaneous bursts of activity also were abolished. The spontaneous bursts reappeared promptly upon cessation of stimulation (fig. 5).

E. *Independence of cortical areas in the production of recruited and spontaneous potentials.* The appearance of recruited potentials in essentially all parts of the cortex after stimulation of localized medial thalamic regions raises the

question of whether the thalamocortical fibers distribute to all parts of the cortex, or whether they project to a localized area which in turn is connnected via intracortical elements with other cortical areas. The following experiments were designed to elucidate this question. The cortex posterior to the anterior suprasylvian fissure was excised, leaving intact the frontal cortex and its thalamic connections. After this procedure, thalamic stimulation was followed by recruited potentials in the remaining cortex. Moreover, spontaneous bursts also were recorded from this isolated piece of frontal cortex. Conversely, in other experiments the frontal cortex was removed from approximately the same level forward, leaving intact the posterior cortex and its thalamic connections. This procedure did not abolish the spontaneous burst, and thalamic stimulation was followed by recruited potentials. Finally, in a last type of experiment, a piece of suprasylvian cortex approximately 1 cm. square was isolated except for its thalamic connections by careful removal of all other cortex in that hemisphere. Even following this radical procedure, spontaneous bursts and recruited potentials were recorded (fig. 6).

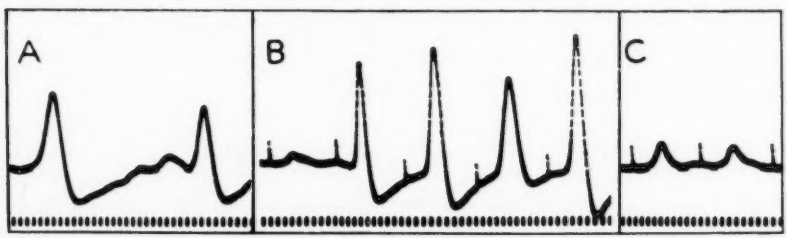

Fig. 6. Bipolar recording, anterior suprasylvian gyrus after removal of surrounding cortex (see text). *A*, spontaneous potentials. *B*, recruited potentials. *C*, potentials remaining at end of series, corresponding to the period of waning response shown in figure 4.

Discussion. A close parallelism in behavior between the spontaneous 8 to 12 per sec. periodic activity and the recruited response is apparent from the data presented in the foregoing sections. The spontaneous bursts may be described as spindles, building up gradually to maximum size and then declining progressively. Similarly, the recruited response increases in size on repeated evocation and later gradually disappears when a continuous series of stimuli is applied (fig. 4). A similarity also obtains between the two in regard to their cortical distribution. Both are of high voltage and relatively simple appearance in the proreus, anterior sigmoid, and middle and posterior suprasylvian gyri, and both are of relatively small voltage and complex appearance in the primary sensory regions located in the posterior sigmoid, posterior marginal and medial ectosylvian gyri (cf. fig. 6, Morison and Dempsey, 1942). Furthermore, both recruited and spontaneous potentials behave alike in that they both appear in small remnants of cortex isolated except for their thalamic connections (fig. 6).

With regard to frequency, it is noteworthy that stimuli applied at a rate slower than the 8 to 12 per sec. frequency of the spontaneous burst usually set up a

train of cortical potentials at this frequency, with the result that the stimuli fall in random fashion among these potentials. On the other hand, stimuli applied at a rate markedly exceeding that of the spontaneous potentials leads to a complete breakdown of both the recruited and spontaneous bursts (fig. 5). Between these two extremes lies a band in which the recruited potentials may be driven accurately at frequencies only slightly greater than those of the spontaneous bursts (fig. 6).

Although the frequency of the potentials cannot be increased markedly above that of the spontaneous burst, it is possible to shorten the period between the bursts themselves. Practically continuous bursts are produced when a few shocks are delivered whenever the spontaneous burst shows signs of failure. Similarly, whenever a continuous series of stimuli is delivered at an appropriate frequency a periodic waxing and waning of the recruited response occurs just as there is a periodic appearance and disappearance of the spontaneous bursts (fig. 4). Moreover, the size of the spontaneous potential evoked by a single shock bears a relation to the phase of spontaneous activity present at the time of the stimulus (fig. 3).

Stimulation at a rapid rate not only fails to evoke the recruited potentials, but also the spontaneous bursts fail to appear. Whatever the mechanism of this effect, it is not due to any durable change produced by excessive stimulation as is shown by the prompt reappearance of spontaneous activity when the stimulation is stopped (fig. 5). This characteristic serves to distinguish sharply between recruiting and primary responses as the latter can follow frequencies up to 120 per sec. (Morison and Dempsey, 1942). Lastly, it has been shown elsewhere that the recruited potential cannot be superimposed upon a maximal spontaneous potential, and that the simultaneous presence of a small spontaneous potential markedly reduces the size of the recruited effect (Dempsey and Morison, 1942). In other words, the two potentials cannot simultaneously coexist, but are blocked, one by the other. It seems justified, because of the above considerations, to regard the recruited and spontaneous potentials as identical.

CONCLUSIONS

Stimulation of medial thalamic regions with a slow frequency series of electrical shocks sets up a recruiting potential of 20 to 35 msec. latency, which is widespread in the cerebral cortex (figs. 1, 2 and 3). This recruiting response is similar to the spontaneous 8 to 12 per sec. potentials in the following respects.

1. The distribution and magnitude of the two potentials are similar (fig. 3; see also fig. 6, Morison and Dempsey, 1942).

2. The recruiting potentials follow accurately at stimulus frequencies near those of the spontaneous 8 to 12 per sec. activity (p. 297). At slower frequencies, trains of 8 to 12 per sec. activity appear which are out of phase with the stimuli (p. 296). Faster stimulus frequencies cause a breakdown of the recruited potential and an abolition of the spontaneous potentials during the period of stimulation (fig. 5, p. 296).

3. The size of the initial recruiting effect is related to the phase of the spontaneous burst at which it is induced (fig. 3, p. 296).

4. Long continued stimulation at appropriate rates leads to a periodic recruitment and de-recruitment similar to the waxing and waning of the spontaneous bursts (fig. 4, p. 297).

5. Both spontaneous and recruited potentials can be recorded from areas of cortex isolated except for their thalamic connections. Neither effect depends, therefore, upon long intracortical connections (fig. 6, p. 298).

6. Recruited and spontaneous potentials show essential similarities in behavior when the interaction of various cortical responses is studied (p. 299, see also Dempsey and Morison, 1942).

Because of the above considerations it is suggested that the recruited and the spontaneous 8 to 12 per sec. potentials are identical.

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THE INTERACTION OF CERTAIN SPONTANEOUS AND INDUCED CORTICAL POTENTIALS

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It has recently been shown that thalamic stimulation gives rise to widespread cortical responses whose appearance and general behavior are similar to those of the 8 to 12 per sec. bursts of activity which are the most striking characteristic of the spontaneous electrocorticogram (Dempsey and Morison, 1942). Further investigation revealed that these potentials could be induced by stimuli applied to the dorsomedial thalamus, while activation of other thalamic regions was followed by different types of cortical responses (Morison and Dempsey, 1942).

The finding that medial thalamic regions were involved in the production of cortical potentials was surprising, since the cortical connections of the intralaminar nuclei are not well established (Walker, 1938). Furthermore, Dusser de Barenne and McCulloch (1938) showed that local strychninization of the sensory cortex led to increased activity in the corresponding part of the ventrolateral thalamic nucleus, and vice versa. It is, of course, well known that the medial lemniscus and spinothalamic fibers end in this nucleus, and that it projects specifically to the sensory cortex. For these and other reasons, Dusser de Barenne and McCulloch (1938a and b) suggested that the spontaneous electrocorticogram results from activity in reverberating circuits between cortical areas and the thalamic nuclei which project to them.

The stimulation experiments mentioned above indicate that the thalamic representation of the 8 to 12 per sec. activity is in the medial thalamus, rather than in the nuclei which project specifically to the cortex. It has been attempted, therefore, to clarify this point by an investigation of the interaction between spontaneous potentials and potentials induced by activation of various thalamic regions.

MATERIAL AND METHODS. Cats, anesthetized with nembutal (0.7 cc. per kgm.) were used. In some experiments deeper anesthesia was required to increase the interval between periodic spontaneous bursts, and in these instances additional nembutal was given intravenously.

The electrical responses of the cortex were led off through bipolar silver-silver chloride electrodes whose separation distance was about 1 mm. Amplification, recording and stimulation were the same as employed in studying the characteristics of the cortical recruited potential described elsewhere (Dempsey and Morison, 1942) and need not be repeated here.

The general procedure employed was to set up a potential in a given cortical

region by stimulation of the thalamus or a peripheral nerve. The size of this potential when induced during a quiet period was compared with its size when superimposed upon spontaneous potentials. Similarly, in other experiments, two potentials, set up by stimuli delivered at two different places, were induced simultaneously and the presence or absence of block was noted.

RESULTS. A. *Interaction of sensory and spontaneous potentials.* Stimulation of a peripheral nerve leads to the appearance of a potential which is localized in the part of the sensory cortex corresponding to the bodily distribution of the stimulated nerve (Marshall, Woolsey and Bard, 1937; Forbes and Morison,

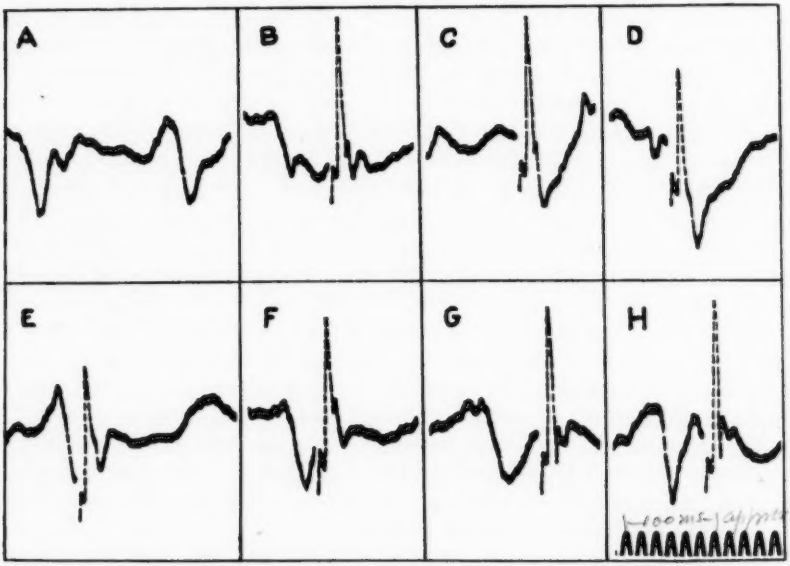

Fig. 1. Bipolar recording, sciatic cortical projection. A, two spontaneous potentials developed during a burst of 8 to 12 per sec. activity. B, cortical response induced by a single shock applied to the sciatic nerve. C, D, E, F, G and H, sciatic responses superimposed without block upon various phases of the spontaneous potentials. Time intervals, 17 msec.

1939; Dempsey, Morison and Morison, 1941). In lightly anesthetized or conscious monkeys, this response ordinarily appears during any degree of spontaneous activity, but occasionally block occurs (Marshall, Woolsey and Bard, 1941).

In cats under nembutal anesthesia it was observed that a single shock applied to the sciatic or radial nerve induced a potential in the corresponding cortical area regardless of the presence or absence of spontaneous 8 to 12 per sec. activity. Likewise, application of a series of stimuli to the nerve during a burst of activity produced as large cortical responses as when the series began during the silent period. Furthermore, the sensory responses could be superimposed upon the spontaneous potentials in all phases (fig. 1).

B. *Interaction of sensory and recruited potentials.* It has been shown elsewhere (Morison and Dempsey, 1942; Dempsey and Morison, 1942) that stimulation of medial thalamic regions gives rise to recruiting cortical responses which on the basis of appearance, behavior and distribution are markedly like the spontaneous potentials. A further similarity became apparent when the interaction between the recruited potential and the sensory response was studied. Since the magnitude of the recruited response depends upon the number and frequency of preceding stimuli used to evoke it, the experiment was performed in the following manner. A series of stimuli at a frequency of 4 or 5 per sec. was applied to the thalamus. Under these conditions it was determined that the maximal recruited potential usually followed either the third or fourth shock. After this had been determined, the series was repeated, and, in addition, a single shock was delivered to the sensory nerve at various intervals before and

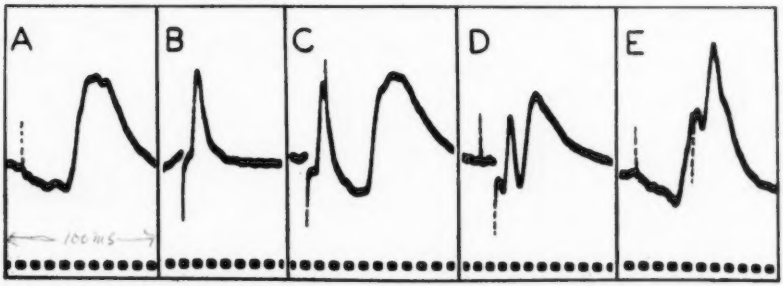

Fig. 2. Bipolar recording, radial cortical projection. A, recruitment response after stimulation of intralaminar thalamus (see text for detail of experiment). B, cortical response induced by stimulation of radial nerve. C, D and E, radial responses superimposed without block upon various phases of the recruited potentials. Time intervals, 10 msec.

after the thalamic stimulus evoking the maximal recruited potential. Figure 2 is the record of such an experiment, and shows that the sensory response may be superimposed upon any phase of the recruited potential.

C. *Interaction of sensory and thalamic "primary" potentials.* Stimulation confined to the nucleus ventralis pars externa of the thalamus gives rise to a potential localized in the sensory cortex which is similar in appearance to, but shorter in latency than, the sensory response recorded after activation of a sensory nerve (Morison and Dempsey, 1942). That this potential induced by thalamic stimulation is indeed the same as that induced by sensory nerve stimulation is shown by the following experiment. Stimulating electrodes were inserted into the ventrolateral nucleus and a potential localized to the leg sensory cortex was recorded after the application of a single shock (fig. 3B). Stimulation of the sciatic nerve likewise produced a cortical response whose appearance was similar except for a slightly longer latency (fig. 3A). Activation of both thalamus and sciatic at short intervals was followed by a single cortical

response, e.g., a thalamic stimulus produced a cortical response, but when the sciatic shock followed soon thereafter the response was almost completely blocked (fig. 3C).

D. *Interaction of recruited and spontaneous potentials.* In lightly anesthetized animals a single stimulus to the medial thalamus frequently induced repetitive 8 to 12 per sec. activity which closely resembled a burst of spontaneous potentials. When a series of stimuli was applied at a frequency slightly slower than the prevailing spontaneous frequency, the stimuli fell random fashion among the potentials of this induced burst. When this occurred, there was complete block of the recruited response. Faster stimulus frequencies (10 to 15 sec.) caused good recruiting potentials in such preparations. This characteristic of the recruitment effect was utilized in an attempt to superimpose the recruiting upon

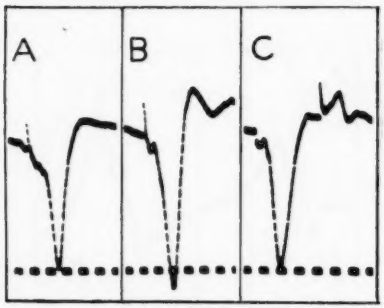

Fig. 3

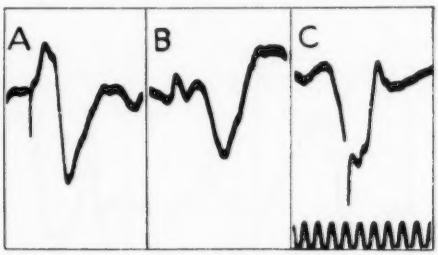

Fig. 4

Fig. 3. Bipolar recording, sciatic cortical projection. A, response to sciatic stimulation. B, response to stimulation of N. ventralis pars externa. C, stimulation of pars externa followed by sciatic stimulation. Note almost complete block of the sciatic response. Time intervals, 10 msec.

Fig. 4. Bipolar recording, anterior sigmoid gyrus. A, recruitment response induced by intralaminar thalamic stimulation. B, spontaneous potential recorded during a burst of 8 to 12 per sec. activity. C, stimulus for recruitment response delivered during the spontaneous potential. Note block of the recruitment response. Time intervals, 17 msec.

the spontaneous potential. At the beginning of a burst of 8 to 12 per sec. activity, a series of thalamic stimuli was delivered at a relatively rapid rate (15/sec.). Since the spontaneous potentials occurred at a slightly slower frequency, there were occasions in which the recruiting stimuli were timed in such a fashion that the two potentials should have occurred simultaneously. Under these conditions one or the other was blocked. When the recruiting stimulus occurred sooner than the spontaneous potential, the recruitment response alone appeared. On the other hand, when the spontaneous potential appeared first, the recruitment potential was blocked to a degree corresponding to the size of the spontaneous potential. When the spontaneous potential was large, there was complete block of the recruiting potential (fig. 4). When the spontaneous wave was small, a recruiting response occurred, but it also was small in comparison to its size when induced in the absence of spontaneous potentials.

DISCUSSION. The method adopted in this study depends upon the assumption that two neuronal impulses cannot simultaneously utilize the same pathway. If, in any two neural circuits, a common path exists at any point, activity in one circuit should render the common path unresponsive for the second circuit. The line of reasoning here is the same as that employed in the concept of the final common path (Sherrington, 1906).

An important corollary to the above is that any two neural potentials which can be induced simultaneously must result from the activity of completely separate elements. Otherwise, unresponsiveness in the common path would block the appearance of one of the potentials.

The applicability of these theoretical considerations to the material at hand is demonstrated in section C. The pathways from peripheral nerves to sensory cortex, according to classical anatomy and physiology, relay from the medial lemniscus through the ventrolateral thalamic nuclei to the cortex. Activation of this pathway at the thalamic level leaves it unresponsive to a subsequent volley set up by peripheral stimulation (fig. 3).

It should be pointed out, however, that complete block does not necessarily occur when two potentials both employ a common neuronal path. The systems dealt with here are multifibered. If, therefore, a submaximal response is evoked, some of the fibers will not be activated and a second response induced from any afferent to this system will find some fibers responsive. Consequently, complete block should occur only when the common pathway is maximally activated, while in submaximal conditions partial block or reduction in size of the response should occur. Block, therefore, represents merely an extreme case of fractionation of a pathway by one of two or more states of activity. That such fractionation normally does occur is indicated by the fact that spontaneous potentials completely block the recruiting response when the spontaneous potential is maximal in size (fig. 4) but not otherwise (section D).

There is a considerable body of evidence available which indicates that spontaneous cortical activity is dependent upon intact connections between the cortex and thalamus. Bremer (1935) found that cortical activity is not altered markedly by transection of the brain stem behind the thalamus. On the other hand, Dusser de Barenne and McCulloch (1938a) showed that the spontaneous electrocorticogram is abolished or abnormal after chronic cutting of the corticothalamic and thalamocortical fibers. Likewise, in unpublished experiments from this laboratory we have observed permanent abolition of the 8 to 12 per sec. activity after removal of the thalamus. These experiments all indicate the importance of the thalamus for the production of the spontaneous electrocorticogram.

The particular thalamic regions which control spontaneous cortical activity are not thoroughly understood. Dusser de Barenne and McCulloch (1938a), on the basis of local strychninization experiments, proposed that closed chain circuits exist between the sensory cortex and the sensory relay nuclei in the ventrolateral thalamic mass (fig. 5A), and that activity within these circuits could explain the spontaneous electrocorticogram.

The experiments reported here, however, demonstrate that spontaneous 8 to 12 per sec. activity and the sensory responses to peripheral nerve stimulation can both occur simultaneously at the cortical level. This observation indicates that the neuronal paths concerned in the production of the two potentials are entirely separate, and therefore the pathway responsible for the 8 to 12 per sec. potentials cannot employ the neurons in the ventrolateral nucleus which relay from the great afferent systems to the cortex. Obviously, the thalamo-cortico-thalamic circuit (fig. 5A), proposed by Dusser de Barenne and McCulloch (1938a), cannot account for the spontaneous bursts of potentials observed during nembutal anesthesia. Indeed, the separability of these two effects has al-

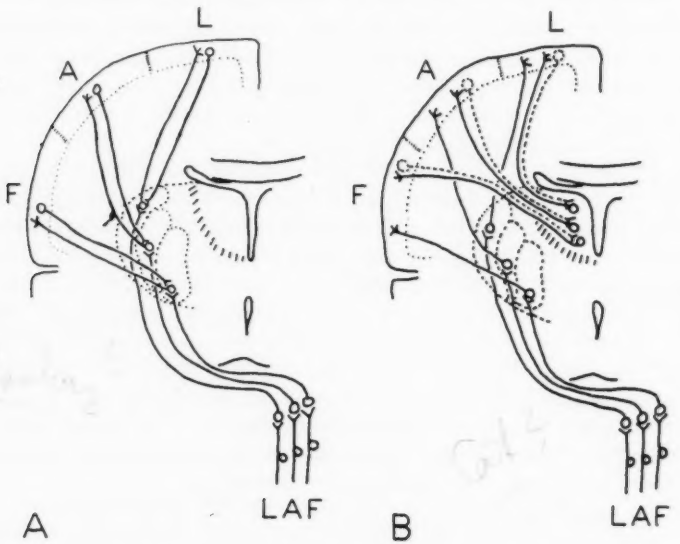

Fig. 5. A, diagram of thalamocortical relations after Dusser de Barenne and McCulloch (1938a). See text for discussion. B, modified diagram showing connections, the evidence for which is presented herewith.

ready been suggested by the suppression of strychnine spikes after degrees of anoxemia which did not abolish spontaneous activity (Dusser de Barenne, Marshall, Nims and Stone, 1941).

It has been shown elsewhere (Morison and Dempsey, 1942; Dempsey and Morison, 1942) that stimulation of medial thalamic regions induces recruiting cortical responses which are quite similar to the potentials of the 8 to 12 per sec. activity. Moreover, the data presented in section D show that the recruiting and spontaneous potentials cannot simultaneously co-exist, but that the presence of one blocks the appearance of the other (fig. 4). Although not conclusive proof, this fact argues for a common pathway serving the recruited and spontaneous potentials. There is, fortunately, other evidence which indicates

the identity of the two effects. The appearance, magnitude, distribution and frequency characteristics of the recruited and spontaneous responses are the same (Dempsey and Morison, 1942), and they show similar interactions with the sensory response (sections A and B, figs. 1 and 2). It seems justified, therefore, to regard the recruited and the spontaneous potentials as being identical. Since the recruited response may be elicited from the medial thalamus, it follows that this region is involved in the spontaneous 8 to 12 per sec. cortical activity.

For the reasons cited above, it is considered that the medial thalamus sends fibers to the cerebral cortex which control one component of spontaneous activity. These connections are shown diagrammatically in figure 5B. For convenience, a single thalamocortical neuron has been represented, but the data by no means imply that direct anatomical connection is essential. Indeed, the long latency of the recruited response suggests that several intermediate neurons may be involved. Furthermore, the anatomical course of the thalamo-cortical fibers is not clear. Several attempts have been made to separate the primary from the recruiting response by lesions placed in the thalamic nuclei and radiations, but the results of these attempts have so far not been conclusive. Consequently, the possibility exists that the fibers for the recruiting response may travel with the radiations from the projection nuclei, although the thalamic cells for the two fiber systems must be different. Different cortical areas each receive thalamo-cortical fibers, because it has been shown elsewhere that the generalized cortical appearance of the recruited response does not depend upon long intra-cortical connections (Dempsey and Morison, 1942). The fibers from the ventrolateral nucleus to the cortex are shown as separate elements, since the records shown in figures 1 and 2 indicate that the sensory pathway to the cortex is always open, regardless of activity in the medial circuits. The corticothalamic neuron which returns to the ventrolateral nucleus, although well known from both anatomical and physiological observations, has not been included, since there is no evidence of its implication in the particular responses studied here.

The type of circuit employed from the medial nucleus to the cortex is not well understood. Because of Dusser de Barenne and McCulloch's demonstration of closed chain circuits between cortex and thalamus, it has usually been considered that spontaneous cortical activity results from reverberating activity in such circuits. The present study indicates that these particular closed chains are not responsible for the 8 to 12 per sec. activity, and reopens the question of the circuit characteristics necessary for such effects. It is of course obvious that reverberating paths could account for rhythmically recurring potentials. On the other hand, there is evidence that nervous tissue may react in a rhythmic manner to constant stimulation in the fashion shown by the well-known rhythmic beat of heart muscle when activated by tetanic stimulation. Repetitive effects have been observed in nerve during and after d.c. stimulation (cf. Rosenblueth, 1941, for references). Consequently, the possibility exists that the 8 to 12 per sec. activity could result from constant bombardment by

subliminal impulses which sum periodically in the cortex. For these reasons the corticothalamic neurons shown in broken lines in figure 5B signify that the circuits have rhythmic discharge characteristics, although the actual presence of a closed chain is hypothetical until further evidence is available.

CONCLUSIONS

1. The potentials evoked in the sensory cortex by peripheral nerve stimulation can be induced at any phase of the spontaneous 8 to 12 per sec. cortical potentials occurring in cats under nembutal anesthesia (fig. 1).

2. The sensory potentials likewise can be superimposed upon the recruiting potentials evoked by stimulation of dorsomedial thalamic regions (fig. 2).

3. The sensory potentials are blocked by concomitant stimulation of the ventrolateral thalamic regions (fig. 3).

4. The recruited potentials cannot be induced in the simultaneous presence of maximal spontaneous potentials (fig. 4) and vice versa.

5. These data show that the spontaneous and the recruited cortical potentials cannot employ the elements in the ventrolateral thalamus which relay the great afferent systems to the sensory cortex. The bearing of these findings on other thalamocortical relationships is discussed.

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EFFECTS OF INORGANIC IONS ON THE RESPIRATION OF BRAIN CORTEX[1]

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The continuation of observations from this laboratory on the respiration of guinea pig tissues in various media has brought to light certain effects of inorganic ions, especially on brain respiration, which we are here reporting. The results refer specifically to the effect of potassium, sodium, lithium and calcium with some incidental observations on the effect of glucose and of total molarity.

METHODS. The oxygen consumption was determined in most cases by means of the direct Warburg method, though in some cases a Warburg-Barcroft differential manometer system was used. The actual period of observation was one hour as a rule, and usually a period of about 1½ hours elapsed from the time the animal was killed and the tissue removed until the end of the experiment. The tissue studied was either guinea pig or rat brain, chiefly cortex, though doubtless some subcortical tissue was included. The tissues were sliced free-hand and kept in a constant volume of control medium until transfer to the respiration flasks. We think it advisable to stress the constancy of the fluid volume in which the tissues are kept after slicing, because it is our experience that laxness in this respect may affect the results, due probably in most cases to washing out of substrate. The pH of the media was determined with a glass electrode before the experiment, and in each respiratory flask after the experiment. This we believe to be an essential check on the buffering capacity of the medium in the face of an active metabolism, being especially important in the case of brain, as we have previously shown (Canzanelli, Greenblatt, Rogers and Rapport, 1939). It also is a check on the ever present danger of splashing, with consequent contamination of the medium with alkali. Failing the actual determinations, we feel that it is unwise to take the pH of even buffered media for granted, and that serious errors in interpretation may result if this precaution is neglected. The pH determinations are omitted for brevity, but all the included results are based on experiments in which a pH was maintained which would not affect O_2 consumption (loc. cit.).

In all cases, unless otherwise stated, glucose was added to the medium in a concentration of 100 mgm. per cent.

RESULTS. *1. The effect of potassium.* The influence of potassium on brain respiration has been observed by Ashford and Dixon (1935) who found a stimu-

[1] Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

lation of rabbit brain respiration with 0.1 M KCl when added to a NaCl-PO_4 mixture. In these experiments the total molarity of the solution was apparently increased. Dickens and Greville (1935) repeated these experiments in rat brain, in a bicarbonate and in a phosphate buffered Ringer's solution, with similar results. Recognizing, however, the considerable hypertonicity of the solution, they substituted KCl for NaCl in four experiments to produce an isotonic solution containing 0.1 M K. In three of these experiments, the respiration was depressed, a result which they attributed to a deficiency of sodium. Our experiments with potassium follow:

a. The effect of varying the molarity of K inversely with Na at normal total molarity. In these experiments the medium was NaCl buffered with a mixture

TABLE 1

QO_2 of guinea pig brain slices in salt media with varying concentrations of K under different conditions

conc. K	K + Na = 0.162 M						Na = 0.162 M		K + Na = 0.303 M	
	Jan. 17	Jan. 15	May 27	May 29	May 29*	Sept. 2	Jan. 17	May 27	Mar. 4	May 29
0	9.7	9.4	9.0	8.4	8.4	10.2	9.7	9.0	2.8	
0.001		10.1								
0.005		13.9								
0.010	16.9	14.8					15.4			
0.020		18.4				20.7				
0.040	22.8	21.1	22.3	21.0	13.8	22.6	21.2	20.3	12.0	11.8
0.060						19.9				
0.080						19.2			15.7	
0.100						17.8				
0.120						13.3				
0.143	10.9	6.5	9.8				15.2	15.0		
0.152									17.1	
0.188										11.1
0.220									12.3	
0.285									6.9	

* In this experiment K and Na = 0.152 M but 0.3 M glucose was added to make the total osmotic pressure equivalent 0.3 M NaCl.

of Na_2HPO_4 and NaH_2PO_4 mixture (which we designate "(Na)PO_4") so that the final phosphate concentration was 0.01 M, and the total molarity equivalent to a 0.15 M NaCl solution. KCl was substituted for NaCl to give the desired molarity of K. The results are seen in table 1. It will be seen that the addition of K to the basic medium became effective in increasing the respiration when the molarity of K reached 0.005 or about the normal K concentration of mammalian body fluids. The effect was progressively greater until 0.04 M was reached, when the O_2 consumption was more than doubled. This concentration was about the same as the optimal concentration of K for pigeon's breast muscle found by Kleinzeller (1940). When the K molarity reached 0.143, or the approximate equivalent of the normal molarity of Na in mammalian serum and tissue fluids, the stimulation was no longer evident. The question arises whether

this is due to excess K or lack of Na, as Dickens and Greville postulated at 0.1 M concentration of K. We will return to this point presently, on the basis of other evidence.

b. Varying the molarity of K and the total molarity, with Na and PO_4 constant. The basic medium was as in (1-a). KCl was added as a solid, thereby increasing not only the K but the total molarity. It will be seen from the experiments in table 1 that with increasing amounts of K there is a progressively greater stimulation of respiration, up to 0.04 M. Unless increased molarity *per se* stimulates the respiration, it is clear that the stimulating K effect seen in section 1-a is due to increased K and not to diminished Na. But increased molarity tends, in itself, to depress brain respiration. For example, doubling the osmotic pressure of a solution by adding glucose to isotonic phosphate buffered NaCl, reduced the QO_2 from 9.9 to 5.0.

c. Varying K inversely with Na at constant high total molarity. The stimulating effect of K in various concentrations is further seen in table 1, last two columns, which summarize experiments carried out at a total constant osmotic pressure equivalent to a 0.3 M NaCl solution. In the experiment of March 4, such a solution containing only Na almost completely inhibited consumption of O_2. As K was increasingly substituted for Na, this depression was converted to stimulation and then finally to depression again. At a K molarity of 0.15, the QO_2 was 17.1 (cf. QO_2 of 15.2 and 15.0 under fairly similar conditions, table 1, cols. 6 and 7); yet at this concentration of K in the presence of a very low Na concentration the QO_2 was considerably less, and in a series of seven experiments in which the K concentration was 0.15 M, with no Na in the medium, the QO_2 was constantly about 30 per cent lower than in a medium containing 0.15 M Na and no K.

Moreover, in a solution with a constant 0.02 M K, 0.01 M PO_4, a varying Na concentration, and glucose sufficient to make the total osmotic pressure equivalent to a 0.15 M NaCl solution, the results seen in column 1, table 2, were obtained. It is evident that with no Na in the medium the characteristic K effect was absent.

The lack of stimulation seen at the higher concentrations of K only appears to occur when the Na concentration is very low, or entirely absent. It might seem as though the stimulating effect of K is counteracted by the depressing effect of low Na. However, as we shall see presently, low Na in itself has no demonstrable effect on brain respiration. Hence we have to conclude that K has only a stimulating action on brain respiration, but this stimulation can only take place, as Dickens and Greville (loc. cit.) postulated, in the presence of an adequate amount of Na. This amount is apparently somewhat in excess of 0.02 M.

It is also true, as in the May 29 experiment in table 1, that the K stimulation can be at least partially counteracted by an increased total osmotic pressure due to added glucose or Na, as will be seen by comparing the effect of a solution of (1) 0.11 M Na and 0.04 M K with (2) 0.11 M Na, 0.04 M K, 0.03 M glucose solution, and (3) 0.263 M Na and 0.04 M K solution, respectively.

The stimulating effect of K on brain is in sharp contrast to its depressing action on nerve respiration, as shown by various workers, and similar in some respects to its effect on muscles (Fenn, 1931; Chang, Shaffer and Gerard, 1935).

2. The effect of Na. We have already seen in the preceding section certain effects that might be ascribed to changes in Na. In these experiments, however, there have been concomitant changes in K. We have studied the effect of changing the concentration of Na in experiments where there was no other cation in the medium, and in which the total osmotic concentration was kept constant with glucose.[2] The results of these experiments are seen in table 2. Clearly the reduction in Na even to 0.02 M had no appreciable effect upon the respiration of the tissue, so long as the osmotic pressure of the solution was maintained. Unfortunately, we found it impossible to obtain results worth recording when hypotonic solutions were used, for when the osmotic pressure fell below the equivalent of a 0.12 M NaCl solution, the tissues tended to disintegrate, so that dry weights were quite untrustworthy and the QO_2 figures obtained were meaningless.

TABLE 2

QO_2 of guinea pig brain slices in salt media with varying concentrations of Na under different conditions

CONC. Na	K = 0.02 M*	Na + GLUCOSE = 0.16 M†				Na + K = 0.16 M			Na + Li = 0.16 M		
	June 24	June 20	June 24	July 23	Aug. 7	June 13	June 19	June 23	June 13	June 19	June 23
0	8.9										
0.02		10.8	8.1	8.7	8.0	8.6	8.1	8.5	16.6	11.8	12.6
0.04	20.4	10.8	10.0	9.8	8.6						
0.06		10.8		10.0	10.3		14.2	12.4		13.9	14.1
0.08	20.5	11.9	12.3	11.7	10.5						
0.10		10.4		10.7	11.2						
0.12	23.9	11.0	10.4	10.2	10.5		20.6	21.7		11.1	13.4
0.15	17.5	11.2	10.6	10.0	9.5		8.8	13.0		10.2	10.6
0.16							8.0	7.4		8.0	7.4

* Media made isotonic by the addition of glucose.

† 0.3 M glucose added to make a total osmotic pressure equivalent to 0.16 M NaCl.

Increasing the Na ion above the physiological level was of necessity associated with increasing molarity, and under these circumstances resulted in an increasing depression of respiration. Thus, with a QO_2 of 8.0 at 0.15 M Na in a NaCl-glucose-PO_4 medium, the QO_2 at 0.18 M Na was 6.5; at 0.2 M Na was 6.3, and

[2] In connection with this, it is well known that in the absence of other substrate in the medium glucose exerts a profound effect on brain respiration. We have performed an experiment in which glucose has been added to NaCl-PO_4 medium to make final glucose concentrations varying from 10 mgm. per cent to 1 gram per cent, the solution being correspondingly hypertonic. At 10 mgm. per cent, the respiration was increased 163 per cent over that in the medium without glucose. This is practically maximal, the changes between this concentration and 1 gram per cent being insignificant. This indicates 1, that above a certain minimum, which we suppose would vary with different animals, the respiration is unaffected by increasing sugar as substrate; 2, that within these narrow limits hypertonicity (equivalent to an increase of 0.03 M NaCl) does not appreciably alter the respiration.

at 0.22 M Na was 4.7. In another experiment, however, there was no difference between the respiration at 0.16 M Na and that at 0.18 M Na, though at 0.14 M Na it was higher. On the whole, it can be said that decrease in the Na ion *per se* does not affect O_2 consumption, and that it is probable, though admittedly not proved, that increase in Na above the physiological concentration depreses O_2 consumption only by virtue of its effect on the total osmotic pressure.

3. The effects of lithium. Our chief purpose in introducing experiments on lithium was to throw light on the potassium effect, but they became of some interest in respect to lithium itself. The results are seen in table 2. Li is capable of stimulating brain metabolism as compared with the level in a solution containing only Na as cation. The highest value for K at 0.04 M is not matched by Li, but at some concentrations, notably at 0.14 M the Li effect is appreciably greater than that produced by K. Li, it is true, can hardly be regarded as of physiological importance. But it was postulated by Dickens and Greville that in stimulating metabolism K was acting in a purely physical way, and was a member of a lyotropic series in which Li was much less active in producing permeability changes and swelling than was K, with Na occupying an intermediate position. At 0.10 M concentration, moreover, they found that Li exerted no appreciable effect on brain respiration. Our experiments with Li do not confirm this; nor is there any evidence in our results that the respective respiratory effects of Li, Na and K are functions of their lyotropic effects.

4. The effects of a balanced inorganic solution including the effects of Ca and Mg. For studying the respiration in a medium containing inorganic ions in approximately their concentration in mammalian serum, we made a modified phosphate buffered Ringer's (MPBR) as follows: 0.137 M NaCl, 115.5 parts; 0.150 M KCl, 4.0 parts; 0.110 M $CaCl_2$, 1.5 parts, and 0.150 M $MgSO_4$, 0.5 part. To this was added when mixed 6.4 parts of "(Na)PO_4" buffer solution. This solution is made by mixing 250 cc. 0.4 M NaH_2PO_4, 235 cc. 0.4 M NaOH and 15 cc. water. The final MPBR solution is 0.15 M with a pH of about 7.5. Its molarity in ions is as follows: Na = 0.143; K = 0.0047; Ca = 0.0013; Mg = 0.0006; PO_4 = 0.010; Cl = 0.131; SO_4 = 0.006.

We have found it possible, by adding the phosphate buffering solution last, slowly and with shaking, to get 5 mgm. per cent Ca into solution. This corresponds roughly to the inorganic Ca, or "the diffusible mols." of mammalian serum according to Flexner (1934).

Five experiments involving the use of MPBR are shown in table 3, and indicate the following:

1. That in a fully balanced medium as to inorganic ions (MPBR) the respiration is the same as in a medium (NaCl-PO_4) in which Na is the only cation.

2. That Ca depresses the respiration, for the respiration in MPBR is higher when Ca is removed. The experiment of March 5 shows that the depression induced by Ca is a function of its concentration. Ca has been variously shown to influence the respiration of different tissues, and with a few exceptions the effect has been one of depression. For rat brain this was shown to be true by Dickens and Greville (loc. cit.).

3. That respiration in MPBR without Ca is practically the same as in NaCl-PO_4 plus K, indicating that the significant inorganic ions in controlling brain respiration are Ca and K.

4. That Ca neutralizes the stimulating effect of K, as shown by the observation in point no. 3 above and that the depression of respiration by Ca is due solely to its abolition of the K effect, because in an MPBR solution containing both Ca and K the respiration is the same as in buffered NaCl and in MPBR containing Ca but no K.

In one experiment the absence of Mg had no influence on guinea-pig brain respiration. In concentrations of 0.10 M, Dickens and Greville found it to depress respiration in rat brain, while Chang, Shaffer and Gerard found it had no effect on dog or rabbit brain. There seems to be a species difference in this case, for in rat brain, as might be inferred from Dickens and Greville's experiment, we found that the respiration in MPBR was increased when Mg was omitted.

TABLE 3

The effects of Ca and Mg guinea pig brain

		NaCl-PO_4		MPBR			
				"Normal"	"Altered"		
		No K	0.005 M K	0.0013 M Ca (5 mgm. %)	0.00065 M Ca (2.5 mgm. %)	No Ca	No K
	1941						
QO_2	Feb. 19		11.8		10.8		
	Feb. 26		13.1		10.4		
	Mar. 5		13.6	8.5	10.4	13.0	
	Mar. 17	10.0		10.0			
	Mar. 19	9.8	13.0	10.0		13.2	9.6
	Aug. 11	10.0	11.8	8.4	9.2	11.3	8.0

On the other hand we cannot confirm the statement of these authors that K is not stimulating when Na is the only other cation in the medium. The data in this paper, including table 3, show that this is not true in guinea-pig brain, and we have also found that adding 0.005 M K to NaCl-PO_4 medium increases the respiration of rat brain as well. Perhaps concentrations have something to do with it.

SUMMARY

The influence of inorganic ions on the oxygen consumption of guinea pig brain in vitro has been studied with the following results:

1. K stimulates respiration in concentrations as low as those present in mammalian serum. The optimum concentration appears to be about 0.04 M. K does not stimulate except in the presence of at least 0.02 M Na. Low Na is not in itself depressing to respiration.

2. Hypertonicity of the medium tends to depress respiration.

3. Li stimulates respiration in concentrations greater than 0.01 M.

4. In a fully balanced medium as to inorganic ions, the respiration is the same as in a solution containing Na as the only cation. This is so because Ca and K appear the only organic cations that exercise a controlling influence on respiration. In depressing respiration, Ca apparently does so solely by inhibiting the stimulating action of K.

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THE EFFECT OF SERUM PROTEINS, AND OF SERUM AND ITS ULTRAFILTRATE ON TISSUE RESPIRATION[1]

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In a previous communication (Canzanelli and Rapport, 1939) this laboratory reported observations on the O_2 consumption of certain guinea-pig tissues in diverse media, the most striking of these being the fact that while the respiration of liver, kidney and heart was higher in serum than in buffer NaCl or Ringer,s solution, that of brain was lower. We are here reporting experiments which were instituted with the purpose of analyzing these phenomena.

METHODS. The methods were similar to those of the paper referred to above. The oxygen consumption of the sliced tissues was determined either in an open Warburg or in a Barcroft-Warburg differential manometer system, usually the former. We have used for comparison with serum and its ultrafiltrate 1, a saline solution buffered with a sodium phosphate mixture (referred to as "NaCl-PO_4"); 2, a saline solution buffered with Na_2HPO_4 and KH_2PO_4 (referred to as "NaCl-Sörensen PO_4") and 3, the modified phosphate buffered Ringer's solution (MPBR) described in the accompanying paper (1941). Horse serum was used and was made almost bicarbonate free by acidifying to a pH of between 6.0 and 6.5, then shaking with evacuation for about $1\frac{1}{2}$ hours, and finally bringing to physiological pH with NaOH. The protein-free ultrafiltrate was prepared by filtering serum under sterile conditions through cellophane (Visking sausage casing $\frac{1}{2}^{s}$" diameter). A few points in regard to the technique we used in this connection may be of value for two reasons: 1, that it made sterility comparatively easy of attainment; and 2, that a large filtering surface could be obtained with the use of a comparatively small amount of fluid. The details of the apparatus are presented in figure 1. The apparatus is sterilized by connecting the glass tip *G* to an ordinary distilling flask from which steam is generated and passed through the apparatus for 10 to 15 minutes, the rubber dam *B* being tucked inside the large cylinder *F*, and a large beaker being inverted over the upper end of the apparatus. After cooling, 50 cc. of horse serum were introduced into glass tube *A*, and the tube then connected at its upper end to an oxygen cylinder as a source of pressure. One to three pounds' pressure was applied, forcing practically all of the serum into a space between the glass tube *A* and the cellophane casing, affording a large filtering surface. The yield

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of ultrafiltrate from 50 cc. serum on overnight standing was usually between 20 and 25 cc.

The ultrafiltrate was made almost bicarbonate free in the same manner as the serum.

The experiments were usually of one hour duration and the pH was noted before and after, experiments in which the pH (as determined by glass electrode) went beyond normal limits for respiration being discarded.

Except where otherwise noted, the respiration of the tissue in NaCl buffered with 0.01 M PO_4 (final concentration) is used as a base in computing percentage changes.

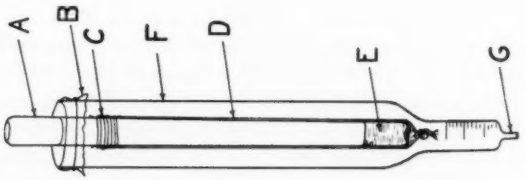

Fig. 1. Ultrafiltration apparatus. *A*, glass tube 18/32 inch diameter, 18 inches long; *B*, rubber dental dam cover to large cylinder; *C*, rubber band sealing upper end of "Visking" casing; *D*, Visking cellophane casing 18/32 inch diameter; *E*, horse serum under 1-3 lbs. pressure; *F*, glass cylinder with tapered and graduated end; *G*, tip for introducing steam to sterilize apparatus and for withdrawal of ultrafiltrate.

TABLE 1

Effect of serum, serum ultrafiltrate and serum proteins on O_2 consumption of liver

TEST MEDIUM	NO. EXPTS.	QO_2		CHANGE
		Control (in NaCl-Sörensen PO_4)	Test	
				per cent
Horse serum	75	3.9 ± 0.1	8.8 ± 0.2	+126
Ultrafiltrate	20	4.0	9.1	+128
Globulin (5 gm.%)	21	3.3	4.7	+42
Albumen (5 gm.%)	6	3.2	4.3	+34

RESULTS. *1. Effects on liver respiration.* In table 1 are given the figures showing that the respiration of liver slices is more than double in serum than in "NaCl-Sörensen-PO_4." (Since these figures were derived from a large number of random experiments, we have determined and stated in the table the Standard Deviation of the Mean.) The possibility existed that this was due to inorganic ions. While we have incidentally observed certain effects on QO_2 as a result of varying the inorganic ions of the medium, a direct comparison of the O_2 consumption of liver slices in the MPBR solution previously referred to and serum, showed the following results: in MPBR $= 3.4 \pm 0.2$; in serum 8.3 ± 0.2. Clearly the serum effect could not be ascribed to any inorganic ion.

The effect of serum proteins was next considered. We prepared serum globu-

lin and serum albumen by precipitation from horse serum three times with half and fully saturated ammonium sulfate, respectively, dialyzing against running water until free of sulfate, washing with ether, and drying by freezing in vacuo. We are not unaware of the fact that we are dealing here with impure products, but with this qualification, we may refer to the fractions we obtained as roughly representing "serum globulin" and "serum albumen."

Table 1 shows the effect of adding each of these proteins to the NaCl-Sörensen-PO_4 medium. In a concentration of 5 grams per cent, or about that normally present in serum, there was in 21 experiments an average increase of 42 per cent in the liver respiration. Except in two experiments, the increase was always unmistakable. In these two experiments, a new batch of guinea pigs from an unusual source were used. A shift to animals from our usual source of supply gave us our customary effect; moreover, livers from animals of the batch in which the globulin effect had failed were found to respond to globulin later, when they had been in the laboratory for some time. We are inclined to believe therefore that a nutritional factor is involved here. The stimulating effect of the globulin was very questionable at a concentration of 0.3 gram per cent; it became evident at 2 grams per cent and maximal at 5 grams per cent. It did not change when the concentration was increased from 5 to 10 grams per cent.

Serum albumen in a concentration of 2 to 5 grams per cent had an effect on liver similar, though somewhat smaller, than serum globulin.

Serum ultrafiltrate. It appeared from the above results that we might be dealing with two distinct phenomena, for the protein effects, even if summated, were not sufficient to account for the effect of the serum. To test this further we used as a medium the ultrafiltrate of serum, prepared as described above, and found in an average of 20 experiments that the ultrafiltrate raised the QO_2 of liver almost exactly as much as did serum itself, namely 128 per cent. It seems clear therefore that the serum effect is quite distinct from the effect of the proteins in the serum, and is due to an as yet unidentified factor.

It is of interest in this connection that Shaffer, Chang and Gerard (loc. cit.) found that respiration of frog and dog liver in coagulated serum and cerebrospinal fluid was as high as in whole serum; and Walthard (1934) found in 3 experiments out of 8 an increased respiration when an alcoholic extract of serum was added to Ringer's solution, while egg white added to Ringer's was without effect. Heating serum to 60° and 65°C. for 15 minutes did not alter the serum effect; nor did similar heating of the ultrafiltrate, or boiling it for 5 minutes with precautions against concentration, change the ultrafiltrate effect.

To find out whether the stimulation due to protein and that due to serum and ultrafiltrate could be summated, we performed 5 experiments, with the results seen in table 2-A. It is clear that when 5 per cent globulin is added to either serum or ultrafiltrate, the effect of the protein is completely abolished. However, it was possible even though unlikely that in these experiments we had reached a "ceiling" beyond which no further stimulation was possible. We therefore performed the experiments in table 2 (B and C). Globulin, to make final concentrations of 2.5 per cent and 5 per cent, respectively, was added to

serum and ultrafiltrate which had been "diluted" with the control solution. Under these circumstances, when it was certain that the ceiiing had not been reached, again there was a complete absence of summation.

When either serum or ultrafiltrate is mixed with NaCl-Sörensen-PO_4 solution in different proportions, and liver respiration compared with that in NaCl-Sörensen-PO_4 alone, the increase in O_2 consumption of the liver was a linear function of the serum or ultrafiltrate concentration, at least up to 80 per cent concentration, as will be seen in figure 2.

The respiration of liver was unaffected by the addition of 100 mgm. per cent glucose to any of the above media.

TABLE 2

Summation of globulin effect and of serum and ultrafiltrate effects on the O_2 consumption of guinea pig liver

Serum and ultrafiltrate

	CONTROL NaCl-SÖRENSEN PO_4	NaCl-SÖRENSEN PO_4 + 5% GLOB.	ULTRAFILTRATE	ULTRAFILTRATE + 5% GLOB.	SERUM	SERUM + 5% GLOB.	
A	3.3	4.8 +40%	8.5 +164%	8.4 +161%	8.0 +143%	8.0 +143%	

Serum

	CONTROL NaCl-SÖRENSEN PO_4	NaCl-SÖRENSEN PO_4 + 2.5% GLOB.	NaCl-SÖRENSEN PO_4 + 5% GLOB.	100% SERUM	25% SERUM*	25% SERUM* + 2.5% GLOB.	25% SERUM* + 5% GLOB.
B	3.1	4.0 +29%	4.7 +52%	8.7 +181%	5.9 +90%	5.8 +87%	6.1 +97%

Ultrafiltrate

	CONTROL NaCl-SÖRENSEN PO_4	NaCl-SÖRENSEN PO_4 + 2.5% GLOB.	NaCl-SÖRENSEN PO_4 + 5% GLOB.	100% U.F.	25% U.F.*	25% U.F.* + 2.5% GLOB.	25% U.F.* + 5% GLOB.
C	3.3	3.9 +16%	5.8 +76%	7.8 +137%	5.1 +55%	5.6 +70%	5.8 +76%

* Serum and ultrafiltrate diluted with NaCl-Sörensen PO_4.

The protein effects on liver metabolism raise the important question as to whether this may not be associated with proteolysis and an amino acid stimulation such as Krebs (1933) obtained in both liver and kidney.

2. Effects on kidney respiration. Kidney slices were chiefly cortical, though medulla was included. By making individual pieces small, pooling was facilitated as much as possible.

In a series of paired experiments, we have observed that the respiration of guinea-pig kidney is on an average 22 per cent greater in NaCl-Sörensen-PO_4 containing 100 mgm. per cent glucose than when glucose is absent. In this respect kidney is, at least in most experiments, unlike liver, which is indifferent to the presence or absence of glucose in the medium. We attribute this to the fact that kidney is lacking in substrate, a lack which glucose supplies. Serum

and its ultrafiltrate stimulate the O_2 consumption equally, as will be seen in table 3, the percentage increase depending upon whether glucose has been added to the control medium. It appears that while serum ultrafiltrate can furnish substrate to the kidney to take the place of glucose, the stimulating

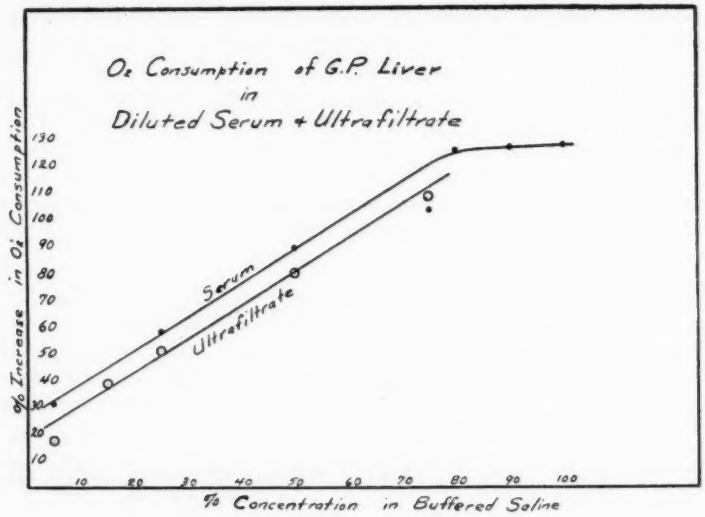

Fig. 2

TABLE 3

Effect of serum, serum ultrafiltrate and serum proteins on O_2 consumption of kidney

TEST MEDIUM	NO. EXPTS.	QO_2				CHANGE
		Controls		Test		
		NaCl-Sörensen PO_4 No glucose	NaCl-Sörensen PO_4 + glucose	No glucose	Plus glucose	
						per cent
Horse serum	8	13.8		22.4		+62
Horse serum	3		15.7		20.9	+33
Ultrafiltrate	6	14.5		23.0		+58
Ultrafiltrate	2		16.6		21.1	+27
Globulin (5 gm.%)	11	12.8		14.4		+13
Globulin (5 gm.%)	3		14.2		14.0	−1
Albumen (5 gm.%)	3	13.5		14.5		+7

effect of these media is due to something apart from this, as in the case of liver. (The apparent depression of metabolism in serum and ultrafiltrate media when glucose is added is factitious, and due to the fact that the results in different animals are used. In paired experiments in tissues from the same animal this

depression is not evident.) As in the case of liver, the serum and ultrafiltrate effects were not due to inorganic ions as shown by experiments with MPBR.

Yamamoto (1938) found that respiration of rabbit kidney was slightly but definitely higher in serum than in Ringer's solution. Karczag obtained astonishing stimulatory effects of human serum ultrafiltrate in guinea pig kidney as compared with the respiration in Ringer-Locke's solution, the control QO_2 being 5.7 (very low in our experience); that in ultrafiltrate being as high as 94.6. We may say in passing that in our hands human serum produces no such effect, but we have not tried the human ultrafiltrate.

As will be seen in table 3, the serum proteins exerted very little effect on kidney. In 11 experiments, the average increase in O_2 consumption upon the addition of 5 per cent serum globulin was only 13 per cent. This is almost within the limit of error, and its significance lies chiefly in the fact that it was inconstant. In 4 experiments there was practically no effect, in 5 others the increase was between 10 and 20 per cent; in the remaining 2 it was 36 per cent

TABLE 4

*Effects of serum and ultrafiltrate on O_2 consumption of brain**

	QO_2				
	Controls			Test	
	NaCl-Sörensen PO_4	NaCl $(Na)PO_4$ + 0.005 M K	MPBR	Serum	Ultra-filtrate
18 experiments	11.5			8.2	
5 experiments	13.0				10.4
February 9		11.8	10.8	10.2	10.6
February 26		13.1	10.4	9.6	10.6

* All media contained 100 mgm. per cent glucose.

in each. We think this is explicable on the basis of the experiment with glucose in table 3. Here the control QO_2 in NaCl-glucose-PO_4 was 14.2; and in the same solution plus 5 grams per cent globulin it was 14.0. Globulin therefore seems to be acting here purely as a substrate, and glucose can substitute for it. The failure of the globulin effect, when it occurred with no glucose added, was probably due to the fact that sufficient substrate was already present in the kidney to obviate the necessity of using globulin. Albumen had practically no effect on O_2 consumption.

3. Effects on brain respiration. We have previously reported (1939, loc. cit.) that the respiration of brain is lower in serum than in both Sörensen-phosphate buffered NaCl and Ringer's solution, containing 100 mgm. per cent glucose. Table 4 shows further experiments, using NaCl-glucose-Sörensen PO_4 as a control, and again the depression due to serum is evident. Moreover, the same phenomenon occurred in the case of the ultrafiltrate. This held true also when the K and PO_4 of the control saline solution was the same as that of the serum. However on making a similar comparison with MPBR, we were unable to dupli-

cate our earlier results with phosphate buffered Ringer's, as will be seen in table 4. Analysis of the discrepancy brought to light the fact that owing to the high molarity of PO_4 used in making the Sörensen buffered Ringer's, ionic Ca of the solution was unquestionably considerably lower than in MPBR and either serum or ultrafiltrate.

Hence we are now of the opinion that the lower respiration of brain in a serum or ultrafiltrate medium as compared with NaCl-Sörensen PO_4 is due to the fact that in the two former the stimulating effect of K is inhibited by Ca, while in the latter it is not. It is, in other words, an inorganic ion effect, whereas the considerable stimulation of liver and kidney respiration are not. There is, on the other hand, no evidence of *stimulation* of brain respiration by either serum or ultrafiltrate. It should be understood that these remarks apply to conditions where the saline media contain glucose. When they do not, the

TABLE 5

*Effects of serum proteins on O_2 consumption of brain**

		QO_2					
		NaCl Sörensen PO_4(0.0026 M K)	NaCl (Na) PO_4 No K	NaCl (Na) PO_4 + 0.005 M K	MPBR		
					Unchanged	No Ca	No K
6 expts.	Control	11.4					
	Control + globulin	9.4					
4 expts.	Control	13.5					
	Control + albumen	8.4					
Mar. 19	Control		9.8	13.0	10.0	13.2	9.6
	Control + albumen		9.1	7.8	7.5	9.1	8.9
June 25	Control		8.1	13.2	8.1		
	Control + albumen		8.0	9.7	8.1		

* All media contained 100 mgm. per cent glucose.

control QO_2 is always very low, and both serum and ultrafiltrate respiration, presumably due to supplying of substrate, is invariably more than 200 per cent higher.

When either serum globulin or serum albumen is added to NaCl-PO_4 solution containing K, the respiration is always depressed, and especially so in the case of albumen. (See table 5.) As will be seen in the two experiments of March 19 and June 25, this depression however does not take place in the absence of K. The depression appears in comparison with MPBR as well, when Ca is absent, and also, apparently, in MPBR when the stimulating action of K is not entirely inhibited by Ca. This at least is the way we interpret the difference in the two results of March 19 and June 25 when MPBR was the test medium. In one case the control QO_2 was 10.0; when albumen was added it was 7.5; in the other the lower control QO_2 of 8.1 was unchanged by the addition of albumen. There

is a rather surprising similarity in the depressing action of protein on brain metabolism and that of Ca, reported in the accompanying paper (loc. cit.). Both seem to depend on an inhibition of K stimulation.

When sugar is not present in the control medium, the low QO_2 (4 or less) is unaffected by the addition of either of the serum proteins, which the brain apparently cannot use as a substrate.

SUMMARY

The influence of horse serum, serum ultrafiltrate and serum proteins on the in-vitro O_2 consumption of guinea-pig tissues has been studied, with the following results:

1. Liver. Serum globulin and serum albumen increase respiration. The stimulating effect of serum is not due to its proteins. Ultrafiltrate stimulates O_2 consumption to the same extent as serum. The effects of serum, ultrafiltrate and proteins are not summated with each other.

2. Kidney. Serum globulin stimulates respiration variably, depending probably on the substrate present in the tissue. Serum and ultrafiltrate increase respiration to an equal extent, part of the effect being due to addition of substrate.

3. Brain. The serum proteins depress brain metabolism. This effect is similar to that of Ca, namely, an inhibition of K stimulation. The brain cannot use serum proteins as substrate. The depression of respiration by serum and ultrafiltrate is apparently caused by Ca.

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THE SPINAL ORIGIN OF THE PREGANGLIONIC FIBERS TO THE LIMBS IN THE CAT AND MONKEY

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The exact preganglionic sympathetic outflow from the spinal cord in man has not been determined by direct methods; the present concept depends upon Langley's (1) description of the preganglionic paths in the cat. Recently, Sheehan and Marrazzi (2) have determined the preganglionic outflow in the monkey by recording the electrical activity of peripheral nerves during stimulation of the ventral roots of the spinal cord. While their method is dependable, it is not applicable to human subjects since it requires the exposure of peripheral nerves for recording. The following experiments were performed to establish the validity of a method that could be used in the operating room for defining the origin of preganglionic fibers in man. The method consists in stimulating ventral roots of the spinal cord and recording changes in skin resistance.

Stimulation of a preganglionic sympathetic nerve causes vasoconstriction, piloerection, and sweating. Of these we have found the last the most easily recorded, since it is attended by a change in the resistance of the skin to the flow of a direct current. This change has been widely used in the study of reflex sympathetic activity. Sympathectomy causes an enormous increase in the resistance and abolishes reflex changes (3, 4).

METHOD. The thoracic or lumbar region of the spinal cord was exposed by a laminectomy while the animal was under ether and nembutal anesthesia. It was found that circulatory failure, which often followed such extensive operative procedures, could be postponed or prevented by keeping the animals warmed with an electric heating pad, and by maintaining an intravenous infusion of 0.9 per cent saline at 0.5 to 1 cc. per minute throughout the experiment. After the dura had been opened, the dorsal and ventral roots on one side were cut close to the spinal cord, and the distal portions tied with a fine silk ligature. The dura about the emerging roots was cut so that they might be elevated from adjacent tissues for stimulation. All the roots exposed by the laminectomy (usually C_8 through T_{12}, or T_8 through L_7) were cut and prepared for stimulation before any recordings were made. This procedure prevented a reflex discharge over intact ventral roots which might have originated in a spread of the stimulus to the spinal cord; it also permitted stimulation of all the roots in rapid sequence. When possible, the stimulations were repeated or the roots of the opposite side

[1] Fellow of the Rockefeller Foundation.

were prepared and stimulated in the same manner. When the lower roots were to be stimulated, the cord was excised, so that the roots of both sides were more available, and the possibility of stimulus escape eliminated.

The stimulus used was a square wave pulse of 16 msec. duration at a frequency of 30 per second. The intensity was 8 volts measured under load. One electrode, a zinc plate, was fastened with a finger stall to the volar surface of the animal's foot, which was cleaned with normal saline and wetted with saturated $ZnSO_4$ solution. The other electrode was fastened to the exposed muscles of the back. By means of the bridge circuit shown in figure 1, changes in

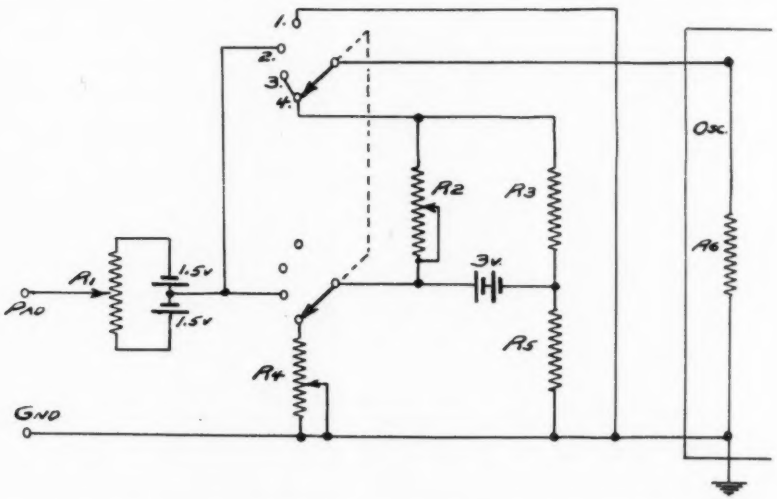

Fig. 1. R_1, 100 ohms; R_2, 100,000 ohms; R_3, R_5, 10,000 ohms; R_4, 100,000 ohm decade resistor; R_6, 0.5 megohm input resistance of oscillograph; *osc.*, 5 inch cathode-ray oscillograph, d.c. coupled, sensitivity approximately 60 d.c. millivolts per inch.

Operation: With switch in position *1*, oscillograph trace is adjusted to a reference position at about mid-screen. With switch in position *2*, R_1 is adjusted to balance tissue potential. With switch in position *3*, R_2 is adjusted to balance the bridge. With switch in position *4*, R_4 is adjusted to again balance the bridge. The setting of R_4 gives skin resistance directly. Recordings were made with switch in position *3*, and calibration was carried out with switch in position *4*.

the skin resistance were recorded on one screen of a dual channel oscillograph; transit time of the electron beam and the stimulus signal on the other. The input circuit does not distinguish between tissue potential changes and changes of potential developed in the bridge due to changes in the skin resistance. Goadby and Goadby (5) have shown that the two are independent and that the former is negligible as compared with the latter. Experiments were carried out to satisfy ourselves that this was the case with the recording system we employed.

Another potential change which must be considered is that due to disturbance

of the electrode during somatic movement. Unfortunately, stimulation of a ventral root may cause movements of the extremity in which changes in resistance are being measured. Two characteristics of the records permit distinction between somatic movement and a decrease in skin resistance: wave form and latent period. Somatic movement begins and ends with the stimulus and the wave fronts are sharp; a decrease in skin resistance has a latent period of about one second and the response is gradual and prolonged.

ROOT	ANIMAL										
	M_2	M_4	M_5	M_6	C_2	C_4	C_5	C_7	C_9	C_{10}	C_{11}
T_1		p		F P p	P		N P	F P N			
T_2	p	p		F P p	P	p	N P	F P N			
T_3	p P	p		P p	P	p s	N P	F P N			
T_4	s 0	p s		P S p	P S	p s	N S P	P N			
T_5	s S	s		S s	P S	p s	S	S			
T_6	s S	s		S	S	s	S	S			
T_7	s S	s		S s	S	s	S	S			
T_8	s 0	s		S s	S	s	S	S			
T_9	s 0	s	o* 0*	S s	S	o	S	S			
T_{10}	o	s	s* 0*	0 s	0		0	0 0*		o*	
T_{11}		o	s* S*					S*		o*	
T_{12}		o	s* S*					S*		s*	o*
T_{13}								S*	o*	s* e	s*
L_1			s* S*					S*	s*	s* e	s*
L_2			s* S*					S*	s*	s* e	s*
L_3			o* S*					S*	s*	s* e	s*
L_4			o* 0*					S*	s*	o*	o*
L_5									s*		

Fig. 2. Summary of positive responses to stimulation of ventral roots in cats and monkeys. E, erection of hair; F, widening of palpebral fissure; N, retraction of nictitating membrane; P, dilatation of the pupil; S, decrease in skin resistance; *, observation made on pad of hind foot; 0, no response. Capital letters indicate a response on the right side; small letters a left sided response.

Both pupils were observed during each stimulation, not only to establish their preganglionic innervation, but to make certain that the stimulus was not exciting the spinal cord. Any response other than ipsilateral dilatation would indicate a spread of stimulus. Observations were also made of retraction of the nictitating membrane, widening of the palpebral fissure, and erection of the hair.

At the end of each experiment, the roots stimulated were identified by dissecting the thoracic nerves into the intercostal spaces. The first lumbar nerve was identified as the second below the last rib.

RESULTS. Positive responses, obtained from 4 monkeys (macasus rhesus) and 7 cats are summarized in figure 2. The cat usually has 13 ribs, the monkey

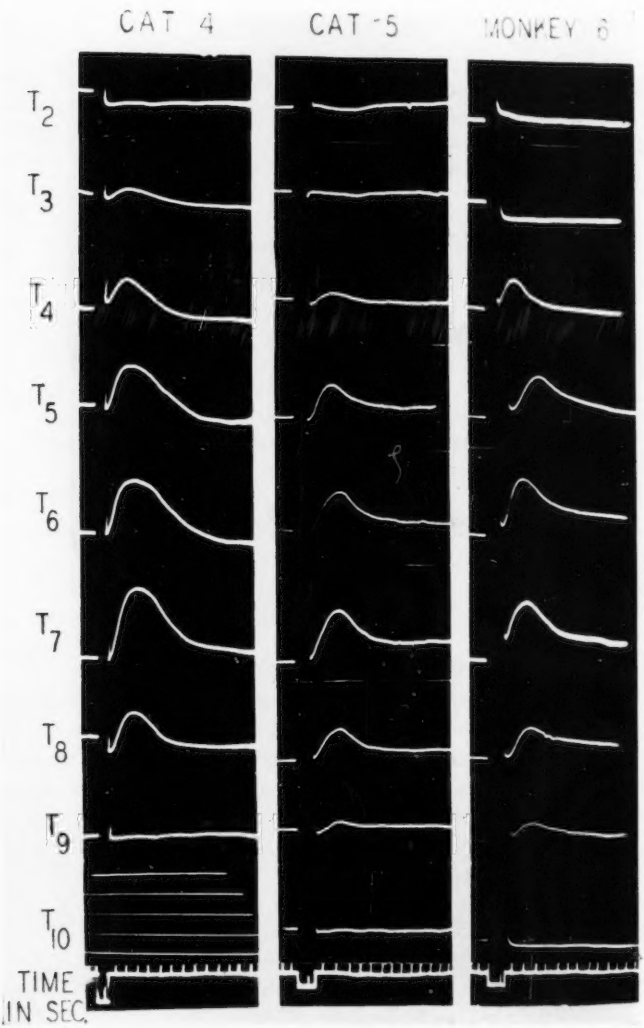

Fig. 3. Oscillographic recordings of the reduction in skin resistance of the forepads of cats 4 and 5 and of monkey 6. The thoracic root stimulated is indicated in the left column. During stimulation the beam was deflected off the screen; an upward deflection signifies a decrease in skin resistance. A sensitivity calibration in 200 ohm steps with a basal of 4900 ohms is substituted for the negative response from T_{10} in cat 4. The basal resistance of cat 4 was 4900 ohms. The bottom record in each column indicates the transit time of the electron beam.

12. Therefore, in figure 2, under T_{13} there are no responses listed in the experiments on monkeys. In the monkey, preganglionic fibers to the eye leave the cord over the first four thoracic ventral roots, while those to the hands emerge through ventral roots T_1–T_{10}. The preganglionic outflow to the monkey's foot, determined in only one animal, was through T_{10}–L_3. One peculiarity in our results is the asymmetry of the preganglionic outflow on the two sides of the same animal. For example, in monkey 5, the preganglionic innervation of the hindlimb was found to leave the cord over the ventral roots of T_{10}–L_2 on the left side and of T_{11}–L_3 on the right. The stimulations were repeated three times with the same results. Another case, this time involving the preganglionic outflow to the hand, is monkey 6. On the right, positive responses were obtained from T_3–T_9 while on the left side the outflow is shifted one segment caudally and includes T_5–T_{10}.

In the cat the preganglionic fibers to the eye are found in the first four or five thoracic ventral roots. The uppermost ventral root influencing the pad of the forefoot was T_5 in 2 cats, T_4 in 2 and T_3 in 1. The preganglionic fibers to the hind foot of the cat extend from T_{11}–L_3, and show considerable variation from animal to animal. In one cat erection of hair on the tail was seen on stimulation of ventral roots T_{13}–L_3.

The changes in skin resistance of the forepad following stimulation of the thoracic ventral roots in cats 4 and 5 and monkey 6 are shown in figure 3. Although the greatest changes resulted from excitation of T_6 and T_7, we have not found it reliable to use the size of the response to estimate the preganglionic content of the ventral roots, since the responses to repeated stimulation of the same root were not sufficiently reproducible.

Discussion. If the stimulus we used was spreading to adjacent roots or to the sympathetic chain, our positive results are of no significance. This possibility was excluded by crushing a ventral root distal to the electrode just after a positive response had been recorded. The stimulus was then repeated and evoked no response. Positive responses in our experiments appear, therefore, to have a real value; negative responses are not so significant since the condition of the animal, depth of anesthesia, or the contact of an electrode may be unsatisfactory and interfere with a response.

The preganglionic outflow determined in these experiments was more extensive than that found by Sheehan and Marrazzi (2). This may be due to the differences of the methods. There is, however, a remarkable agreement on the major outflow and we consider the confirmation of their results evidence for the validity of our method.

Sheehan and Marrazzi are inclined to disbelieve that in man there is a preganglionic outflow to the hand over the upper thoracic ventral roots (6). Their reluctance to accept this idea is based upon generalizations drawn from the studies on the cat, dog, and monkey. In these animals the forelimb receives no preganglionic innervation from T_1 and T_2, and there is only an occasional contribution from T_3 in the cat. By stimulating ventral roots in man,[2] we have

[2] These experiments were done with Dr. Bronson Ray and will be reported elsewhere.

demonstrated a preganglionic outflow to the hand as high as T_2 and as low as T_9.

SUMMARY

By recording changes in skin resistance on stimulating the ventral roots of the spinal cord, the preganglionic sympathetic outflow to the fore and hind feet in the cat and monkey has been determined. This method of determining the preganglionic outflow is applicable to man.

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EFFECTS OF HEXYLRESORCINOL AND OTHER AGENTS ON THE ABSORPTION OF SUGARS, CHLORIDE AND SULFATE FROM THE ALIMENTARY TRACT[1]

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The intent of the investigations reported here was to try to gain some knowledge of the factors which control the absorption of a few simple substances.

Absorption of Syrups and Sugars. a. Absorption of sugars with time. The evidence that there is a special mechanism for the absorption of certain sugars is considerable but inconclusive. A good argument for a special mechanism would be the successful demonstration of a constancy in absorption with time and independently of concentration after solutions of sugar had been placed in the alimentary tract, a constant rate indicating, of course, that a special mechanism is at work constituting the limiting factor. Evidence for such an active cellular function was furnished in 1929 by Cori, Cori and Goltz (1) who claimed that the absorption of glucose was constant from hour to hour, and within wide limits independent of the strength of the solution used. This work was confirmed by Trimble, Carey and Maddock (2), but refuted in 1941 by Nutter and Murlin (3).

The problem of absorption of sugars with time was reinvestigated using the method of Cori (4) for absorption and Bertrand's method for sugar analysis. A falling off in absorption with time was found. A fundamental objection to the original observations of Cori, Cori and Goltz is that 2 cc. of a 50 per cent solution of glucose put into the rat's stomach causes, by its osmotic effect, such a distention that the stomach is unable to empty promptly. Furthermore, it is necessary to exercise the utmost caution to prevent loss by spurting when the stomach is opened. Even with such precaution absorption of sugars as a straight line function of time never has been obtained in these laboratories.

b. Effect of hexylresorcinol, pinacol and calgon. It has been found that hexylresorcinol, pinacol (tetramethyl glycol) and possibly calgon (sodium hexametaphosphate) (5) promote the absorption of insulin from the alimentary tract and an investigation was made to determine the factors responsible for this increased absorption. The results were of sufficient interest to encourage an examination

[1] Most of the data in this paper are taken from a thesis presented in partial fulfillment of the requirements for the Doctorate of Philosophy, University of Rochester, 1940.

of their effect on the absorption of syrups and sugars. The syrups used were "sweetose" and corn syrup, the compositions of which follow.

	Sweetose[2]		Corn syrup[2]	
Moisture	18.05%		19.70%	
Sugars	55.73	81.67	27.70	80.05
Dextrins	25.94		52.35	
Ash	0.28		0.25	
	100.00		100.00	

The Cori technique (4) again was used but a 2 cc. solution equivalent to 25 per cent of glucose was used instead of a 50 per cent solution. The osmotic effect still was apparent, but spurting was not difficult to control. The effect of hexylresorcinol on the absorption of corn syrup, sweetose, glucose and sucrose and the effect of pinacol and calgon on the absorption of corn syrup were studied. The results expressed in absorption per unit weight and body surface are given in table 1. A 25 per cent solution of sweetose is absorbed more rapidly than a solution of corn syrup of the same concentration. This finding can not be explained by the fact that sweetose is more hydrolyzed than corn syrup because glucose gives about the same value as corn syrup. Magee and Reid observed that 13.5 per cent is the optimal concentration for the absorption of glucose in rats and rabbits (6), and since it is the concentration in the intestine next to the mucosa that really matters it is quite possible that 25 per cent corn syrup does not furnish enough glucose while 25 per cent glucose furnishes too much.

The important point in this work is the inhibition in absorption of corn syrup, sweetose and sucrose but not of glucose by hexylresorcinol. In other words, in each case where an enzymatic hydrolysis is concerned, absorption is inhibited by hexylresorcinol and the observation (5) that this compound inhibits the activity of pepsin, trypsin and erepsin is recalled. Pinacol which had no influence on any of the proteolytic enzymes (5) was without effect and calgon, which only checks the activity of pepsin, had, if any, a slight favoring effect on the absorption of corn syrup (table 1).

Absorption of Chloride and Sulfate. a. The chloride impoverishing mechanism in the small intestine. In 1934 Burns and Visscher (7) found that with solutions containing both sulfate and chloride, the latter was absorbed against a high concentration gradient. Ingraham, Peters and Visscher (8) continued the study so far as to allow a theoretical interpretation. Their hypothesis was that an essentially mosaic pattern of differential permeability exists in the intestine. By assuming that monovalent ions move out of the intestine but not into it and that polyvalent ions do not move at all, an equation was derived to describe quantitatively the concentration of the monovalent ion in the loop at any time during absorption. These workers removed samples from the intestinal loop for analyses at various intervals by means of a needle and syringe. This procedure was tried in our laboratory but was modified for two reasons. 1. The volume of the contents of the loop decreases rapidly with time if left undisturbed

[2] Supplied by the A. E. Staley Manufacturing Company, Decatur, Illinois, to whom the author is indebted for some financial aid in this portion of the study.

and then a further decrease in volume is caused by the removal of a sample for analysis. This would seem to preclude a crucial experiment as volume is an important determinant. 2. It was desired to use a technique by which handling of the loop could be avoided.

In the experiments reported here a 75 cc. solution of a mixture of equal volumes of isotonic sodium chloride and sodium sulfate was introduced into the lower twelve inches of the ileum of the dog and removed after a certain interval for analyses. Then a fresh solution was introduced for the next trial, etc. Chloride was determined by the Volhard-Harvey titration method and sulfate by the method described by Koch (9).

TABLE 1

Absorption of sugars in one hour

EXPT.	SUGAR	NO. RATS	AV. WT.	AMT. FED	AMT. ABS. PER 100 GM. BODY WT.	COEF. OF VARIA.	MGM. ABS. PER CM^2 BODY SURFACE	COEF. OF VARIA.
			gm.	*mgm.*	*mgm.*			
I	25% corn syrup	9	147	519	246	10.5	1.708	10.5
II	25% sweetose	9	126	495	323	17.9	2.090	11.8
III	25% glucose	8	125	522	248	19.4	1.602	15.8
IV	25% sucrose	8	119	484	259	24.9	1.628	20.6
V	25% corn syrup; 0.1% hex. res.	9	130	506	198	14.5	1.600	11.1
VI	25% sweetose; 0.1% hex. res.	9	117	552	252	13.4	1.313	13.1
VII	25% corn syrup; 0.1% pinacol	9	110	475	209	24.9	1.286	22.2
VIII	25% corn syrup; 0.1% calgon	7	117	509	286	20.6	1.730	21.9
IX	25% glucose; 0.1% hex. res.	9	116	508	257	21.4	1.472	14.3
X	25% sucrose; 0.1% hex. res.	7	136	418	160	11.1	1.063	11.2

It must be pointed out that all of these experiments deal with apparent absorption because the secretion that is going on at the same time as the absorption was not taken into account.

The equation of Ingraham et al. (8) describing the concentration of chloride in an ileal loop is

$$C = C_0 \left(\frac{V}{V_0}\right) \frac{Ri}{D}$$

where V_o = the original volume of the solution in liters,
C_o = the original concentration of sodium chloride in the solution in millimoles per liter,
V, C = the volume and concentration at any time in the same units,
R_i = the rate of flow of pure water into the intestine from the blood in liters per hour and
D = the rate of volume decrease in liters per hour.

Table 2 was compiled in order to test the validity of the equation. In some cases another substance was added to the salt solution and these are also indicated in the table.

Considerable difficulty was encountered in choosing a value for R_i. In one paper (8) Ingraham, Peters and Visscher stated that this value ranged between 0.150 and 0.250 l. per hour, but in another paper (10) Ingraham and Visscher claimed that the value ranged around 0.030 l. per hour. Using their own data from the latter paper R_i was calculated to be 0.250 l. per hour. This value for R_i as well as 0.025 and 0.100 was used and compared in table 2.

TABLE 2

Test of diffusion theory for chloride absorption

ADDITIONAL SUBSTANCE PRESENT	t	Vo	V	Co	C				
					Ri in l/hr.			Observed	$C = KV$ $K = 800$
					0.250	0.025	0.100		
0.005M	*hours*	*liters*	*liters*	*mM/l.*	*mM/l.*	*mM/l.*	*mM/l.*	*mM/l.*	*mM/l.*
	½	0.075	0.042	76	7.7	61.0	31.3	36.7	33.6
	½	0.075	0.040	76	7.6	60.5	30.5	33.3	32.0
	½	0.075	0.071	76	14.0	63.6	38.4	54.1	56.8
Quinine	½	0.075	0.057	76	11.5	62.9	35.7	63.9	50.6
	½	0.075	0.045	76	7.8	61.3	33.3	46.2	36.0
	½	0.075	0.064	76	11.8	63.1	36.0	81.9	51.2
	½	0.075	0.053	76	10.8	62.4	34.7	49.8	42.4
Calgon	½	0.075	0.052	76	11.0	62.1	35.0	48.1	41.6
	½	0.075	0.061	76	11.8	63.1	36.0	47.0	48.8
Calgon	½	0.075	0.058	76	11.1	62.5	35.0	43.9	46.4
	½	0.075	0.041	76	7.7	61.0	31.4	45.6	32.8
Aerosol O.T.	½	0.075	0.069	76	7.7	74.6	70.6	63.9	55.2
	½	0.075	0.065	76	7.7	74.8	73.3	63.6	52.0
Pinacol	½	0.075	0.063	76	7.7	74.6	70.6	49.5	50.4
	½	0.075	0.055	76	10.4	62.1	34.4	42.5	44.0
Methyl salicylate	½	0.075	0.059	76	12.2	63.2	35.4	47.0	47.2
	½	0.075	0.056	76	11.4	62.8	35.3	41.7	44.8
Quinine	½	0.075	0.060	76	11.9	63.1	36.0	46.2	48.0
	1	0.075	0.029	76	1.6	51.8	16.3	19.8	23.2
	1	0.075	0.027	76	4.6×10^{-1}	45.6	9.9	21.4	21.6
	1½	0.075	0.018	76	6.2×10^{-3}	23.6	1.8	11.9	14.4

Calgon = sodium hexametaphosphate.
Aerosol O.T. = dioctyl sodium sulfosuccinate.
Pinacol = tetramethyl glycol.

It is evident that the concentration of chloride in ileal loops is brought to a very low level and this part of their findings is confirmed. An investigation, however, of their mosaic theory indicates that the phenomena of absorption and secretion are far too complicated to make their simple assumptions. It is true that a rough parallelism was found between the volume of the solution in the loop and its chloride concentration. In these experiments the following simple equation was found to be even more accurately supported by the data.

$$C = KV \text{ where } K = 800.$$

A test of this equation is also given in table 2. From the equation, however, no theory concerning a mosaic structure and differential permeability can arise. It is purely empirical and gives no hint as to the mechanism of absorption.

b. The effect of various substances on the absorption of chloride and sulfate. The consensus of opinion among physiologists seems to be that water and electrolytes are absorbed from the intestine by simple osmosis and diffusion. That there is a special mechanism, however, for the absorption of these substances, as well as for organic molecules, was pointed out by Peters and Visscher (15). Using D_2O and NaCl they found that the former leaves the gut in the direction of, and the latter against the direction of their respective diffusion gradients at rates which approach equality. Since NaCl should diffuse more slowly than D_2O it was inferred that the two are moved by the same mechanism.

In an attempt to find out something about this mechanism, use was made of a number of compounds which influence the absorption of insulin from the intestine (5). The procedures employed were exactly the same as those previously described with the exception of the addition to the solution of the test substances.

TABLE 3

Absorption in one-half hour of chloride and sulfate (in millimeters) from the lower ileum of the dog

X	NO. OF EXPTS.	Cl NORMALLY ABSORBED	Cl ABD. WITH *X*	SO_4 NORMALLY ABD.	SO_4 ABD. WITH *X*
0.005 hex. res.	7	2.51	0.27	0.77	1.56
0.005 NH_4SCN	6	2.45	1.09	0.57	0.47
0.005 aerosol O.T.	6	2.46	1.01	0.66	0.74
0.005 pinacol	6	2.43	2.33	0.66	0.54
0.005 calgon	6	2.58	2.64	0.85	0.83
Sat'd methylsalicylate	6	2.43	2.42	0.66	0.87
Sat'd quinine	6	2.43	1.59		

It is to be remembered that the imposition of extraneous factors on absorption is purely of an exploratory nature, and, until more is known about normal action, the data obtained are extremely difficult to interpret. A reference to table 3, however, brings out at least one definite fact: hexylresorcinol inhibits the absorption of chloride and promotes the absorption of sulfate. Since hexyl reorcinol inhibits the action of pepsin, trypsin, and erepsin (5), is it possible that it inhibits some organic factor responsible in part at least for the movement of chloride to and from the mucosa? If so, could the factor be carbonic anhydrase?

An attempt was made to answer this question by poisoning carbonic anhydrase with ammonium thiocyanate. It can be seen from table 3 that this compound cuts down the absorption of chloride by more than 50 per cent. The thiocyanate was introduced into the loop along with the salt solution, and due account was taken for the amount left in the loop by a colorimetric determination.

On the other hand it must be remembered that Höber, Andersh, Höber and Nebel (14) found that hexyl resorcinol depolarized membranes of muscle and

nerve and postulated that the compound brought about a structural rearrangement of the surface-membrane. It is possible that this rearrangement accounts in part for a decreased chloride absorption.

Quinine, which has a poisonous effect on protoplasm, cut down the absorption of chloride, and Aerosol O.T., a surface tension lowering agent, also decreased absorption but not nearly so much as hexylresorcinol.

The reason for an increased sulfate absorption in the presence of hexylresorcinol is even more difficult to explain. On the basis of osmosis it can be argued that a high chloride concentration would thermodynamically predispose a compensatory removal of sulfate. But, out of forty-seven freezing point determinations, forty-four showed that the net transfer of substances in mols was from the blood into the loop. It is evident, therefore, that simple diffusion also will not explain the movement of sulfate.

Enzyme studies. In view of the finding that hexylresorcinol inhibits pepsin, trypsin and erepsin and the possibility that it may influence absorption by this action, it was decided to test the effect of the compound on steapsin, the sac-

TABLE 4

Effect of hexyl resorcinol and ammonium thiocyanate on enzyme activity

ENZYME	SUBSTANCE ADDED	% INACTIVATION
Steapsin	Hex. res.	0
Amylopsin	Hex. res.	98
Diastase	Hex. res.	96
Invertase	Hex. res.	0
Carbonic anhydrase	Hex. res.	75
Carbonic anhydrase	Am. thiocy.	100

charide-splitting enzymes and carbonic anhydrase. The results are given in table 4 along with the effect of ammonium thiocyanate on carbonic anhydrase.

Discussion. The studies on syrup and sugar absorption bring out several points in regard to the factors which control it. The lowering of surface tension will not bring about an increase in absorption of glucose as was shown by the ineffectiveness of hexylresorcinol, which lowers the surface tension of water from 73 to 27 dynes/cm. As a matter of fact, this compound caused an inhibition in most cases. This inhibition was always present where an enzymatic hydrolysis of the sugar or syrup was concerned. However, in one case hexyl resorcinol inhibited the absorption of sucrose, but in vitro studies show that it does not interfere with the activity of invertase. As was concluded before, (5) hexylresorcinol must have some effect on absorption aside from its inhibitory action on enzymes.

Pinacol, another compound with a hydrophobic and a hydrophilic group, does not influence the absorption of corn syrup. It has already been shown (5) that these groups must be specific for each type of molecule to have any effect on absorption, and the present observation is a confirmation of this finding.

It was somewhat of a surprise to find that calgon was without influence in

corn syrup absorption. This compound is very effective in removing calcium ions (11) which, according to Gardner and Burget (12) inhibit the absorption of glucose. However, McDougall and Verzár (13) using a special diet reduced the blood serum calcium of rats to 60 per cent of the normal amount and found no change in the absorption of glucose. The results reported in this paper confirm the latter finding even though the experimental techniques were quite different.

The studies on chloride and sulfate absorption confirm the observation of Burns and Visscher (7) that chloride, in the presence of a polyvalent anion, leaves the gut against a considerable concentration gradient; but the data do not allow an agreement with the differential permeability theory of Ingraham, Peters and Visscher (8). On the other hand, it appears that some special biological agent or structural arrangement is responsible for the absorption of electrolytes as well as for other substances.

SUMMARY

The absorption of sugar falls off with time as determined by the Cori technique, but it is doubtful if this method comes close enough to a normal physiological condition to place much value on the data obtained.

Lowering the surface tension will not promote the absorption of glucose.

Hexylresorcinol inhibits amylase and diastase of malt but not invertase.

The removal of calcium ions in the intestine does not affect the absorption of glucose.

In the presence of the divalent sulfate ion, the univalent chloride ion rapidly leaves the intestine.

The data do not agree with the theory of Ingraham, Peters and Visscher on the absorption of electrolytes.

The concentration of chloride in the intestine runs parallel to the volume of the solution which contains it if the sulfate ion is present.

Hexylresorcinol, ammonium thiocyanate, aerosol O.T. and quinine inhibit the absorption of chloride, while the former increases that of sulfate.

Hexylresorcinol and ammonium thiocyanate inhibit the activity of carbonic anhydrase in vitro and both also lower the absorption of chloride. It would seem, therefore, that there is a special mechanism for at least a part of chloride absorption and that carbonic anhydrase may be involved, along with a certain structural arrangement favorable for the absorption of the ion.

The absorption of sulfate is likewise partly dependent upon some biological agent not yet investigated.

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REVERSALS OF BLOOD PRESSURE RESPONSES CAUSED BY CHANGES IN FREQUENCY OF BRAIN STEM STIMULATION[1]

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Kabat, Magoun and Ranson (1935) found that punctate stimulation of the interior of the hypothalamus with current from an inductorium regularly caused a rise in blood pressure and regarded this as evidence that the hypothalamus contained a sympathetic center. Similar stimulation of the forebrain immediately in front of the hypothalamus regularly caused falls in blood pressure. Stimuli of the same kind applied to certain definite parts of the medulla caused rises in blood pressure and when applied to other parts of the medulla caused falls (Wang and Ranson, 1939). For convenience of reference we shall refer to the regions, yielding significant rises in blood pressure under the conditions of the experiments mentioned, as pressor areas. However, the recent work of Hare and Goehegan (1941) and of Bronk, Pitts and Larrabee (1940), showing that a change in frequency of stimulation can alter the direction of blood pressure change, resulting from activation of a single point in the forebrain, requires a reconsideration of the relation of both frequency and location of stimulation to the direction of the vasomotor responses.

METHODS. Brief electrical pulses of constant form from the transformer output of a relaxation-oscillator (containing a 0.05 mfd. condenser which discharged through an 884 tube tripped at 200 volts and through a 200 ohm potentiomer tapped by the primary of a General Radio 578-A, air-core transformer) were delivered at rates between $\frac{1}{2}$ and 300 per second through bipolar nichrome electrodes ($\frac{1}{2}$ mm. separation) oriented within the brain by means of a Horsley-Clarke instrument. With this instrument the electrodes could be accurately placed and then held rigidly at one point while the responses from that point were tested with various rates of stimulation. In each brain the reactions from many points were recorded.

Simultaneous oscillographic and kymographic records were made of the cardiovascular responses. Cathode-ray oscillograms of the electrical activity of the left inferior cardiac nerve were made after the nerve had been exposed by resection of the upper left ribs. The arterial blood pressure was recorded from the carotid or femoral artery by mercury or membrane manometers. Such records were made during stimulation of the hypothalamus in 22 cats and of the

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medulla in 16 additional cats. In 28 others only oscillograms were made. In these, single or double shocks were delivered to the hypothalamus or medulla to study the characteristics and after-effects of the induced activity as recorded from the left inferior cardiac nerve. In 16 of these animals, the sciatic nerve was also stimulated with single and double pulses. In these experiments photographs were made of single sweeps which were synchronized with stimuli by trip circuits.

Either chloralosane or urethane was employed and the dosages varied to give light or deep anesthesia in order to rule out the depth of anesthesia as a significant factor in the results. Following each experiment, the cat was perfused with 10 per cent formalin, the brain removed, and the portion used in the experiment (forebrain or medulla) prepared for microscopical study and localization of the points stimulated.

Results. *Two types of reversals of blood pressure responses.* Throughout most of the hypothalamus proper stimulation at rates of 5 or more per second and at any effective voltage (usually above one volt) resulted in a rise in arterial blood pressure. Stimulation of the same points, without moving the electrodes, produced falls when the rate was below 5 per second, providing the voltage was sufficiently high, 10 peak volts or more as measured on the oscillograph. With lower voltages no depressor reactions could be elicited from these points. We shall refer to these as low frequency reversals because the change in direction of response occurred at the low critical frequency of about 5 per second. Figure 1 A shows that stimuli at 24 peak volts and a rate of 42 per second applied to a point in the hypothalamus caused a sharp rise in blood pressure and that at the same point stimuli at the same voltage but at a rate of only 4 per second produced an almost equally sharp fall. On repetition of these tests with lower voltages the same differences in response reappeared. The latent periods were short for the depressor as well as for the pressor responses.

Low frequency reversals were also obtained from those parts of the medulla which, according to Wang and Ranson (1939), consistently yielded pressor responses when stimulated with an inductorium. They resembled in every respect the low frequency reversals obtained from the hypothalamus. Frequently, rises of more than 60 mm. Hg were converted into falls of more than 20 mm. Hg. Figure 2 shows that with stimuli at 20 peak volts and at frequencies of 14, 26 and 45 pressor responses were obtained which increased stepwise with increasing frequency. When the same point was stimulated at the same voltage but at rates of 2.8 and 2.4 per second, falls in blood pressure promptly occurred.

A different type of reversal was obtained from regions bordering on the hypothalamus and from parts of the medulla surrounding the pressor areas. Since the critical frequencies, at which these changes in direction of the vasomotor responses occurred, ranged between 10 and 150 per second, they may be designated as high frequency reversals. Figure 1 B shows that a point just in front of the hypothalamus in the preoptic region yielded a slight fall in blood pressure when stimulated with 32 peak volts at a rate of 14 per second. The same point with stimuli at the same voltage but at rates of 28 and 42 per second gave rises in

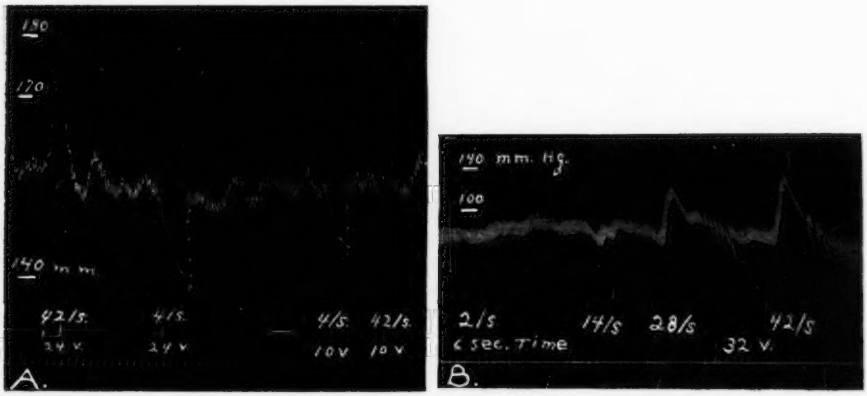

Fig. 1. Two kymograms of the blood pressure responses elicited by stimulation at various frequencies of two regions of the forebrain. A. Low frequency reversals obtained from a point within the hypothalamus 0.5 mm. left of the midline immediately dorsal to the mammillary bodies. From this point pressor responses were elicited with frequencies above 5 per second and depressor responses were found with frequencies below 5 per second. B. A high frequency reversal obtained at a critical frequency between 14 and 28 per second from a point in the preoptic region 1 mm. to the right of the midline just rostral and ventral to the anterior commissure. Tracings were made with a membrane manometer and time was in 6 second intervals.

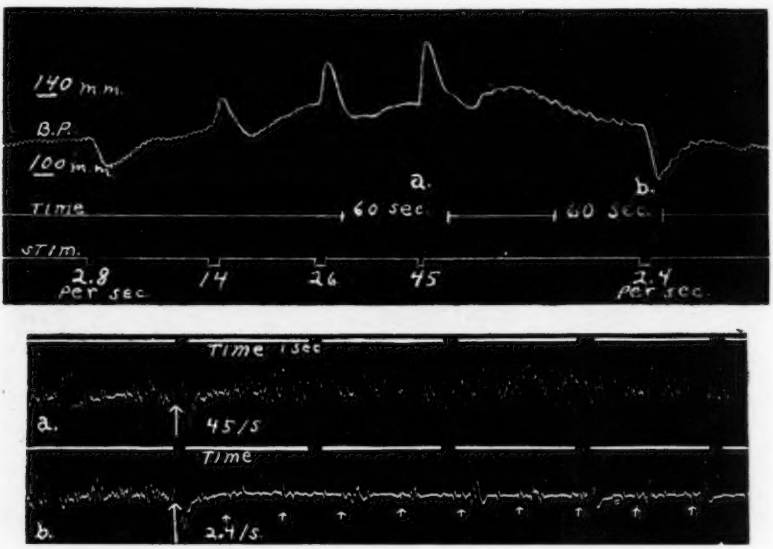

Fig. 2. A low frequency reversal elicited from a point in the reticular formation medial to the nucleus of the spinal tract of the fifth nerve and ventral to the tractus solitarius at the level of the rostral half of the inferior olivary nucleus. The kymogram shows the blood pressure responses to various frequencies of stimulation at 20 peak volts intensity. The continuously recorded oscillograms *a* and *b* show the electrical activity in the left inferior cardiac nerve taken simultaneously with the early phases of the responses at *a* and *b* of the kymogram. In the oscillograms the onset of the stimulation is indicated by a large arrow; in *b* each stimulus is marked with a small arrow. Blood pressure tracings were made with a mercury manometer.

blood pressure. The critical rate in these high frequency reversals was subject to considerable change with slight shifts in the position of the electrode or with changes in the intensity of stimulation. The intensity required was not fixed within any definite range and varied from point to point from below one to 10 or more peak volts; however, the intensity and the critical frequency were not independent, and it was noticed that an increase in stimulus intensity lowered the critical frequency. These reversals could be produced by changes in voltage as well as by changes in frequency. Figure 3 shows that a point in the medulla

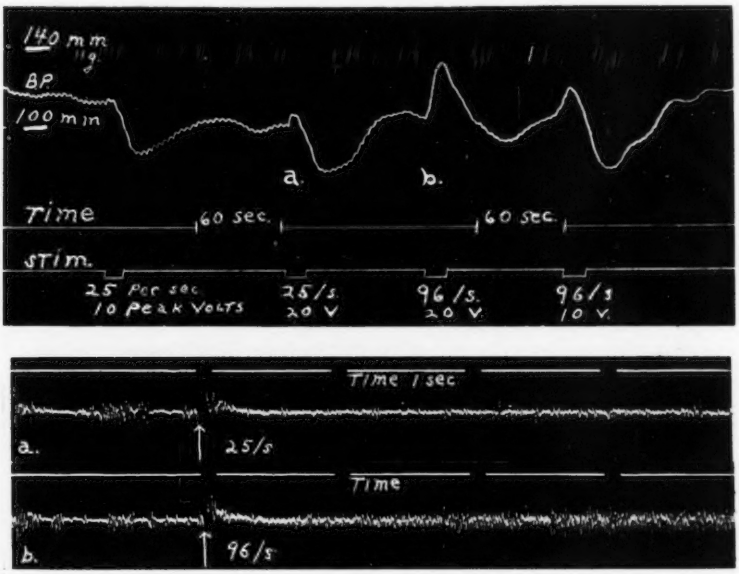

Fig. 3. A high frequency reversal elicited from a point in the medulla 1 mm. to the left of the midline and immediately dorsal to the inferior olivary nucleus at the middle of its rostro-caudal extent. The kymogram shows the blood pressure responses to various frequencies and intensities of stimulation. The continuously recorded oscillograms, *a* and *b*, are records of the electrical activity in the left inferior cardiac nerve taken simultaneously with the early portions of the depressor and pressor responses at *a* and *b* of the kymogram. The onset of stimulation is indicated by large arrows in the oscillograms. The electrical activity throughout the entire stimulation period of *b* continued to increase as it does during the first 4 seconds.

near the midline and immediately dorsal to the inferior olivary nucleus yielded a change from a depressor to a pressor response produced by shifting the frequency from 25 to 96 per second at 20 volts. This reversal could be duplicated by changing the voltage from 10 to 20 volts while fixing the rate at 96. The depressor response to stimuli of 20 volts at 25 per second was complicated by a brief initial pressor deflection which was not elicited by stimuli of 10 volts at the same frequency. The initial pressor deflection was also introduced by changing the frequency from 25 to 96 at 10 volts. In this respect the high

frequency reversals differed from the low frequency reversal in which change of intensity altered only the magnitude of the response and not its direction. The initial rise which in some cases preceded the fall caused a lag in the appearance of the depressor reaction.

Location of points from which each of the two types of reversals were obtained. On drawings of two representative sections through the forebrain the low frequency reversals have been plotted as solid circles the size of which represents the extent of the fall in blood pressure (fig. 4). High frequency reversals have been plotted as solid squares. Solid triangles indicate points which gave only

Fig. 4. The distribution of blood pressure reversals from the forebrains of 22 cats. *A* is a drawing of a representative section in the plane of the Horsley-Clarke instrument at the level of the tuber. *B* is a similar drawing at the level of the anterior commissure. The solid circles show low frequency reversals, the solid triangles indicate high frequency depressors, and the solid squares show high frequency reversals. All reversals represented by circles or squares contain pressor phases greater than 10 mm. The magnitude of the low frequency reversals is indicated by 3 sizes of circles indicating 3 ranges graded with respect to the size of the depressor phase. Thus the ranges are from 1 to 9 mm. Hg, 10 to 19 mm., and 20 mm. or more. The magnitude of the low frequency depressor responses is indicated by triangles of 3 sizes representing depressor responses of the same ranges indicated for low frequency reversals.

depressor reactions with stimuli within the ranges of strength and frequency employed in these experiments. For brevity these will be referred to simply as depressor reactions or depressor points.

Points yielding low frequency reversals were distributed widely through the hypothalamus proper but not in the surrounding structures. Stimulation of these points at rates above 5 per second yielded pressor responses and their distribution corresponds fairly closely with the pressor areas described by Kabat, Magoun and Ranson (1935).

In the medulla oblongata points yielding low frequency reversals were limited to those parts of the reticular formation of the medulla which were designated

by Wang and Ranson as pressor areas. In 16 cats 83 points yielding such reversals were obtained at levels between the rostral and caudal ends of the inferior olivary nucleus. The surrounding structures in the medulla such as the olive, restiform body, vestibular nuclei and medial lemniscus did not give such reversals.

From areas surrounding the hypothalamus anteriorly (preoptic region), dorsally (thalamus), laterally (internal capsule), and ventrally at the surface of the brain, depressor responses were obtained with stimuli at rates relatively high with respect to 5 per second and usually throughout the entire frequency range of the stimulator. But at a few points in these regions surrounding the hypothalamus the falls could be converted to rises in blood pressure by increasing the rate of stimulation (critical frequency 10 to 150 per second). As shown in figure 4, these high frequency reversals occurred at points closely associated with others from which only depressor reactions were obtained. Like the depressor reactions, the high frequency reversals were obtained from points anterior to the hypothalamus and from other points in areas surrounding the hypothalamus but never from the hypothalamus proper. The number of points yielding high frequency reversals was small in comparison to those giving low frequency reversals.

In the medulla depressor responses and occasional high frequency reversals were elicited by stimulating points surrounding the area from which pressor reactions and low frequency reversals were obtained. Thus depressor reactions were obtained from the spinal vestibular nucleus, the olive, and the medial lemniscus. At a few of the depressor points raising the frequency of the stimulation to even higher rates caused the blood pressure to respond with a rise, thus constituting a high frequency reversal. At all of those reversal points tested at the boundaries of the medullary pressor regions increasing the intensity of the stimulation produced reversals similar in all respects to the effect of increasing the frequency. Similar intensity reversals were also found in the hypothalamus.

Electrical activity of the inferior cardiac nerve during reversals. The records of the electrical activity which were taken during the low frequency reversals from the hypothalamus and medulla showed changes in the level of activity in the left inferior cardiac nerve which were associated with the blood pressure responses. With stimulation at rates above 5 per second the rise in blood pressure was accompanied by a corresponding increase in both the size and frequency of the electrical fluctuations (fig. 2 a). The fall in blood pressure with the lower stimulus rate was associated with a general decrease in the activity (fig. 2 b). Although the general appearance of the record during the depressor phase was that of lower activity, it was noticed that the decrease in activity consisted of an abolition of the tonic impulses or normal activity while each stimulus, with the exception of the first few, was followed by a spike which presumably represented an accelerator impulse. At critical frequencies near 5 per second, the appearance of the induced spikes gave the impression that they compensated for the loss of tonic activity.

The oscillograms associated with the high frequency reversals from areas sur-

rounding the hypothalamus and from the medulla showed an increase in activity during the rising phase of the reversal (fig. 3 b), and a general decrease during the falling phase (fig. 3 a).

The results of single or double stimuli to either the medulla, hypothalamus, or sciatic nerves are illustrated in figure 5. With a single strong shock, a large spike of about 200 microvolts' amplitude appeared in the inferior cardiac nerve about 75 msec. after stimulating the medulla and about 100 msec. after stimulating either the hypothalamus or the sciatic nerve. Certain other features were found consistently. The level of activity in the nerve before the passage of the spike was lowered below the level of detection for about 500 msec. during the interval immediately following the appearance of the spike (fig. 5, A, C and E). When a second stimulus was introduced (fig. 5, B and D), the magnitude

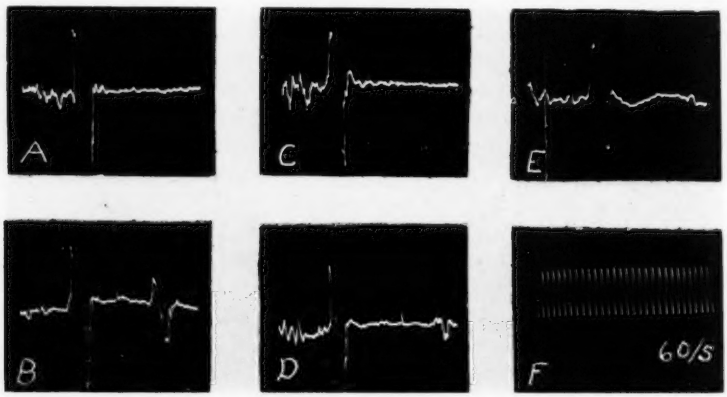

Fig. 5. Single sweep records of the electrical activity of the left inferior cardiac nerve during single and paired stimuli. Single stimuli at 10 volts were delivered to the lateral hypothalamic area at the level of the tuber in *A*, to the sciatic nerve in *C*, and to the reticular formation of the medulla in *E*. Paired stimuli separated by 210 msec. were delivered to the hypothalamus in *B* to the same spot as in *A*. Similarly, *D* was recorded while 2 stimuli separated by 280 msec. were delivered to the sciatic nerve. Time as 60 cycle waves is shown in *F*.

of the induced spike varied with the time separation between the stimuli. Starting at the end of the absolutely refractory period at about 70 msec. with either hypothalamic or medullary stimulation or at about 160 msec. with sciatic stimulation, the magnitude of the second response recovered at a logarithmically decreasing rate. Thus, a single strong shock to either the medulla, hypothalamus or sciatic had a long lasting disruptive effect upon either tonic or artificial excitatory influences.

Reversal of pupillary responses. Frequency reversals of the pupillary responses were elicited from the forebrain and midbrain, but these were limited to a few regions adjacent to those concerned with the light reflex (pretectal region, optic tract and chiasma). Throughout the rest of the hypothalamic structures no such conversions were found. The critical frequencies were varied and high and

were greatly influenced by the intensity of the stimulation which suggests a parallel with the high frequency blood pressure reversals which were also found in only a few limited regions with high critical frequencies and with intensity playing a large rôle in the conversion. Further data concerning the pupillary reactions elicited from the fore- and midbrain have been published by Hodes and Magoun (1942).

Discussion. Inspection of the records published by Hare and Geohegan (1941) indicates that they obtained reversals of the high frequency type from the border of the hypothalamus or from structures surrounding it. From their conclusions one may infer that the number of points, which they found yielding this type of response, was not very large. They did not report low frequency reversals. In fact the voltage of their stimuli was too low to produce them. The record published by Bronk, Pitts and Larrabee (1940) seems to be that of a typical low frequency reversal obtained from the lateral hypothalamic area.

The location of the points yielding low frequency reversals in the pressor areas of the hypothalamus and medulla, and of those yielding high frequency reversals in the surrounding areas, reinforces the evidence for the specificity of these pressor areas. The fact that under the special conditions described a depressor response can be obtained from such an area does not invalidate this specificity.

The electrical records, both continuous and with single sweep, suggest that the falls in blood pressure associated with low frequency reversals are the result of the disruptive effect of the stimulus upon the tonic activity of the cardiovascular nerves, which masks the excitatory effects of the stimulus, if the rate of stimulation is low enough. However, the disruptive effect need not be assumed to occur at the point stimulated but may manifest itself lower in the sympathetic pathways, perhaps at the spinal centers.

Since the high frequency reversals from falls to rises in blood pressure, obtained from points around the pressor areas, can be elicited by increasing the strength of the stimulus as well as by increasing the rate, it seems probable that a spread of excitation to the pressor areas may be responsible for the pressor responses. Although it may be true that the spread of voltage remains unchanged through changes of the rate of stimulation, the possibility that the effective physiological spread through a center may be different at various frequencies of stimulation has not been ruled out. Indeed, the increased response to higher frequencies might indicate that the changes invoked by intensity increase may be mimicked by frequency increase.

SUMMARY

1. Two types of blood pressure reversals were found on stimulation of either the hypothalamus or medulla. Low frequency reversals which showed a conversion from a fall to a rise in blood pressure occurred when the rate rose above 5 per second. High frequency reversals occurred at critical stimulus rates between 10 and 150 per second.

2. Within the forebrain, low frequency reversals were distributed within the

boundaries of the hypothalamus proper while high frequency reversals were limited to structures closely surrounding the hypothalamus anteriorly, dorsally, laterally and ventrally.

3. Within the medulla oblongata at levels of the inferior olive, low frequency reversals were distributed within the reticular formation while high frequency reversals were limited to structures such as the medial lemniscus, the vestibular nuclei, the restiform body, and the inferior olivary nucleus.

4. Electrical records taken during the reversals showed that the direction of the blood pressure response was related to a rise or fall in the activity of the inferior cardiac nerve.

5. Single sweep electrical records with single or paired stimuli to the hypothalamus, medulla or sciatic nerve not only showed the excitatory spike elicited by the stimulus, but demonstrated the disruptive effect of a strong shock upon the background activity, either normal or induced, in the inferior cardiac nerve.

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THE ALTERED FUNCTION OF THE GALL BLADDER OF THE PREGNANT GUINEA PIG

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Numerous cholecystographic studies in pregnant women have shown, for the most part, a delay or incompleteness in emptying of the gall bladder (cf. Schaefer 1933 for references). Moreover, in a beautifully executed series of experiments, Gerdes and Boyden (1936, 1938) found normal gall-bladder emptying during the first trimester, a marked retardation in evacuation during the latter two-thirds of pregnancy, and a return to normal *post partum*.

The factors which bring about this slowing of gall-bladder evacuation during pregnancy are not well understood. Mann and Higgins (1927) reported that the gall bladders of pregnant animals failed to empty in response to a fat meal. Since insertion of paraffin into the uterus after the termination of pregnancy did not prevent a return to normal gall-bladder evacuation they concluded that the effect was not caused by mechanical obstruction of the biliary tract. Smith, Pomarane and Ivy (1941) found a diminished response of the gall bladder when cholecystokinin was injected into pregnant guinea pigs, and that evacuation returned to normal a few days *post partum*. However, *in vitro* experiments showed essentially no difference in response when the gall bladders of non-pregnant, pregnant and experimentally injected animals were tested.

The purposes of the present investigation were to examine the sex hormones as causes for the failure of evacuation and to search for the site of action of the inhibitory factors.

METHODS. Mature female guinea pigs weighing 550 to 850 grams were used. These were divided into five groups: 1, normal, non-pregnant females; 2, pregnant females; 3, females 1 to 5 days *post partum*; 4, ovariectomized females; and 5, ovariectomized females injected with estrin (Progynon B, Schering), or progesterone (Proluton, Schering), or both.

The animals were given no food for 24 to 48 hours before use. Under dial anesthesia (0.6 cc. per kgm. intraperitoneally) a tracheal cannula for artificial respiration was inserted, a femoral vein was prepared for injection, and the gall bladder was exposed by a midline abdominal incision. Animals in which the gall bladder was contracting or was not well filled were discarded. Altogether, 73 satisfactory experiments were done.

The effect of a test dose of 1.0 mgm. cholecystokinin (S.I. of Greengard and Ivy, 1938) was then observed. The degree and speed of contraction were estimated from measurements of the principal diameters of the gall bladder and

from gross appearance. During complete contraction of a well-filled gall bladder the fundus decreases from about 1.5 to 0.5 cm. in diameter. At the same time, the smooth translucent wall becomes opaque and finely nodular as areas of contraction develop and merge. Blood vessels, originally nearly straight, become increasingly tortuous. It is relatively easy, therefore, to recognize a contraction which is delayed in onset or incomplete in extent. In the present experiments responses are classified as "poor" when less or slower than that seen in the majority of control animals. In the remainder the response was classified as normal.

After observation of the initial contraction to a test dose of cholecystokinin, the common bile duct was cannulated in 20 animals from the various groups. A no. 17 transfusion cannula with a metal bead near the tip was inserted through a nick in the duct and tied in place with a silk ligature. The cannula was connected through a 3-way stopcock to a water manometer and to a 5 cc. graduated pipette. The pipette was held in a horizontal position. As the bile flowed outward, its rate of flow could be read in hundredths of a cubic centimeter per minute. By raising or lowering the clamp holding the pipette, the pressure in the system could be adjusted to any desired level. In the present experiments the pressures employed were between 4.5 and 6.0 cm. of water, as recommended by Doubilet and Ivy (1938).

We were unable to occlude the numerous hepatic ducts without either manipulating the gall bladder unduly or interfering with the flow of bile from the cystic duct. Consequently, there was a constant flow of hepatic bile into the pipette, to which was added the flow of cystic bile when contraction of the gall bladder occurred. It was found, however, on clamping the cystic duct and recording only the hepatic bile, that its rate of flow did not change appreciably when cholecystokinin was injected. It seems reasonable to assume, therefore, that the increased rate of flow observed when the cystic duct was open represents quantitatively the added component from the gall bladder.

The chief advantage of cannulation is that it permits study of the gall bladder and the sphincter of Oddi separately without many of the practical and theoretical objections to *in vitro* experiments. A further advantage is that quantitative estimates of gall-bladder responses are possible. Since in the present experiments, however, a test dose of cholecystokinin always was given prior to cannulation, it was felt that the possible "priming" effect of the initial injections might obscure the quantitative aspects of the results. Nevertheless, in each group of animals, data from cannulation experiments were qualitatively consistent with data obtained by previous direct observation of the gall bladder in the same animal.

Results. A. *Normal control animals.* In 12 of the 14 animals in this group the gall bladder contracted normally following a single injection of cholecystokinin. No pathology was evident in these animals at autopsy. In the remaining two, contraction was poor. One of the latter animals had large ovarian cysts, multiple cystic tumors in the uterine wall, and a large fungating tumor in the stomach. The other animal whose response was poor had a gall bladder larger than any other we have ever seen in a guinea pig. It was flaccid, it ex-

tended far down into the right flank, and showed little change in volume even after repeated injections of cholecystokinin.

B. *Pregnant animals.* In the 15 animals of this group contraction was normal in 5 and poor in 10. At the end of each experiment the uterus was opened and the length of the fetuses measured. The duration of pregnancy was then estimated from the table given by Draper (1920) which correlates fetal length with the duration. The 5 animals whose gall bladders had contracted normally contained fetuses 1.8 to 31.0 mm. long, indicating pregnancies of 14 to 32 days. The remaining 10 animals contained fetuses 32 to 123 mm. long, indicating pregnancies of 33 to 64 days.

C. *Post partum animals.* Two animals, 24 and 30 hours *post partum*, respectively, showed poor emptying of the gall bladder. Two other animals, tested 5 days *post partum*, showed normal gall-bladder evacuation when cholecystokinin was injected.

D. *Ovariectomized control animals.* The 11 animals in this group were ovariectomized several days to several weeks before experiments were performed. Five of the group received daily subcutaneous injections of 0.5 cc. cottonseed oil for 10 days. All 11 showed normal gall-bladder evacuation when cholecystokinin was injected.

E. *Ovariectomized, sex-hormone injected animals.* 1. *Estrin.* Four animals received single injections of 50 (2 animals), 500 or 1000 I.U. estrin (Progynon B, Schering) one to three weeks after ovariectomy, and were tested 24 to 60 hours later. Five other animals received 7 or 8 daily injections of 50, 500 or 1000 I.U. and were tested the day following the last injection. Of the 9 animals, 3 showed poor emptying. There was no correlation between dose and degree of emptying, as one animal received a single injection of 50 I.U., the second a single injection of 1000 I.U., and the third 7 injections of 500 I.U.

2. *Progesterone.* Nine ovariectomized animals received daily injections of 0.05 to 0.80 mgm. progesterone from 6 to 36 days. They were tested the day of the last injection. Of the 9 animals 4 showed poor evacuation and 5 were normal. As in the group injected with estrin, little correlation existed between effect and dose. Of the 4 animals showing poor evacuation one received 0.05 mgm. for 6 days, one 0.10 mgm. for 6 days, one 0.40 mgm. for 14 days and one 0.80 mgm. for 11 days.

3. *Estrin and progesterone.* Six ovariectomized animals received daily injections of 10 I.U. estrin and 0.05 mgm. progesterone for 1 to 9 weeks. Three of the 6 animals showed poor evacuation and 3 were normal. Five additional animals were injected daily with 50 I.U. estrin and 0.20 mgm. progesterone for 3 to 7 weeks. All 5 showed poor emptying.

Discussion. The present experiments demonstrate that the decreased gall-bladder motility of pregnancy can be reproduced in non-pregnant animals by suitable injections of ovarian hormones. This demonstration provides additional evidence that the altered function of the gall bladder of pregnancy is caused by humoral rather than mechanical factors. It is of interest that the gall bladder shows changes in response to the estrin and progesterone treatment comparable to the changes known to occur in the uterus and ureters. The

question is raised, therefore, whether the reactions of smooth muscle in general are changed by sex hormones.

The deficient gall-bladder evacuation found in pregnant guinea pigs confirms the findings of Smith, Pomarane and Ivy (1941) and is comparable with the results of Gerdes and Boyden (*loc. cit.*) in their study of pregnant women. Since retardation in emptying is observed after injection of cholecystokinin, it seems clear that the failure must be in the utilization rather than the production of the intestinal hormone. Moreover, the site of failure would seem to be in the gall bladder itself, since slowed and deficient contractions were observed after cannulation of the common duct, thus eliminating the sphincter of Oddi from consideration. The possibility suggested by Smith, Pomarane and Ivy (1941), that inactivation of cholecystokinin in the blood stream might be speeded by altered metabolism of the sex-hormone action, cannot be excluded, however, without experimental proof.

The partial biliary stasis which occurs during pregnancy results in a gall bladder filled with concentrated bile. Little is known about the effect of the bile composition upon gall bladder motility. Naturally, a concentrated viscous bile might cause poor emptying, but this effect is a secondary rather than primary disturbance. Moreover, it has repeatedly been observed in the present experiments that the refilled gall bladder shows deficient contractions to a second or third injection of cholecystokinin.

CONCLUSIONS

1. A method for the cannulation of the common bile duct of the guinea pig is described.

2. Evacuation of the guinea pig gall bladder after intravenous injection of cholecystokinin is markedly diminished in rate and extent during the second half of pregnancy and for a short time *post partum*.

3. A similar deficiency in evacuation occurs in ovariectomized guinea pigs after treatment with estrin and progesterone.

4. The possible regions which participate in the gall-bladder failure are discussed.

We wish to express our appreciation to Dr. E. W. Dempsey for his encouragement and advice throughout these experiments. We also wish to express our thanks to Dr. A. C. Ivy for generously supplying us with cholecystokinin, and for his directions as to its use.

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THE EFFECT OF SMALL LESIONS OF THE ORGAN OF CORTI ON COCHLEAR POTENTIALS[1, 2]

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The resonance theory of hearing, as developed and elaborated by Cotugno, Corti, Hensen, Helmholtz and others, postulates that analysis of sound into its component frequencies occurs in the cochlea, each tone stimulating only a specific part of the auditory end-organ. The clinical and experimental evidence that has accumulated from many sources proves that the receptors for high tones are confined to the basal turn of the cochlea, but leaves unsettled, among others, the following questions: *a*. Is there localization for low tones? *b*. Exactly where and how large are the areas of response for each tone? *c*. What is the effect of an increase in intensity of a tone on the extent of the area of response? The experiments described in this paper were undertaken in an attempt to obtain further information on these questions.

Many investigators have maintained that if the resonance theory of hearing is correct, destruction of a portion of the cochlea should produce deafness for the tones specific to the destroyed area. However, the experimental studies of cochlear localization that have been reported are not in agreement with each other. The differences are explicable, in part at least, as failures to restrict the lesions of the organ of Corti to the part supposedly injured. Therefore, we have given special attention to this problem and have developed a technique with which we have repeatedly been able to destroy the organ of Corti in selected regions without traumatizing other areas or without causing any escape of endolymph. The latter point is extremely important in obtaining consistent results, because simple puncture of the cochlear duct in the basal turn causes a marked impairment of cochlear potentials for all frequencies.

MATERIAL AND METHODS. Cats anesthetized with phenobarbital-sodium were used in all experiments reported in this paper. After tracheotomy, the bulla was exposed by separating the structures ventral to it, and the middle ear opened by cutting away the bulla and removing the septum.

In making the experimental cochlear lesions it is necessary to work under a binocular dissecting microscope. With a small dental burr the bone is removed over the selected part of the spiral ligament, which is then further loosened by

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gentle pressue with a blunt needle. The latter procedure causes the basilar membrane in this restricted region to bend, and the organ of Corti resting on it to be crushed or dislodged. In successful experiments neither Reissner's nor the basilar membrane is torn; there is no escape of endolymph and no hemorrhage into the cochlear duct (fig. 1).

Pure tones, generated by a continuous-sweep, beat-frequency oscillator and a moving-coil receiver, were used as stimuli. The frequency range used was from 32 to 10321 cycles per second. The tones were conducted from the speaker to the cat's ear by $\frac{3}{4}$-inch rubber hose.

Cochlear potentials were used to measure the response of the ear. To pick up the changes in potentials a silver wire was placed on the bone near the lateral margin of the round window niche and a silver bar was inserted into the exposed neck muscles. The potentials were passed through a transformer-coupled amplifier and a band-pass filter, and converted into sound in a head-phone.

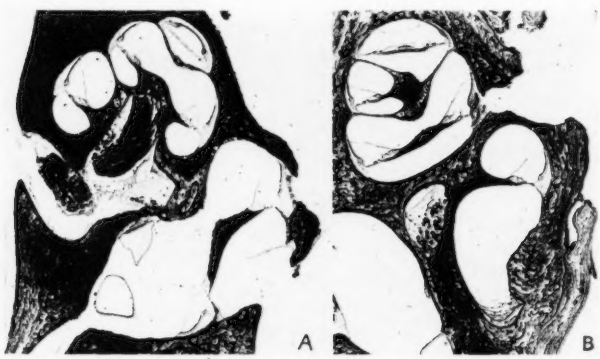

Fig. 1. Photomicrographs of sections of cochleae to show nature of the lesions made. A, lesion in lower basal turn; B, lesion in middle turn.

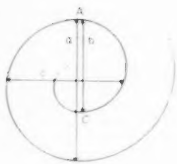

Fig. 2

The threshold of response for each frequency was obtained by increasing the intensity of the stimulating tone in steps of five decibels until the amplified cochlear response was just audible to an observer with good hearing. With the amplification used, threshold responses for normal cats are at intensities of the stimulus tones very close to human thresholds. Cats whose control tests showed impaired thresholds or who had middle-ear infections were rejected. The limits of error of the method are about five decibels.

For each cat the first readings obtained are used as "normal", and changes during the course of an experiment are recorded in decibels above or below this value.

Each ear was histologically prepared and carefully studied to determine what lesions were actually produced. The graphic reconstruction method of Guild (1) was used to determine the location and extent of each lesion. Some of the cochleae were not sectioned exactly in the mid-modiolar plane, and for these it

was necessary to apply a correction factor to the graph. The correction factor was obtained as follows: Except for the vestibular part, the orthoprojection of a cochlea is sufficiently close to a spiral that only slight error is introduced by the assumption that the distance $\frac{a+b}{2}$ should equal distance c (fig. 2). When there is obliquity of sectioning, this distance is less than c. Distance c is measured

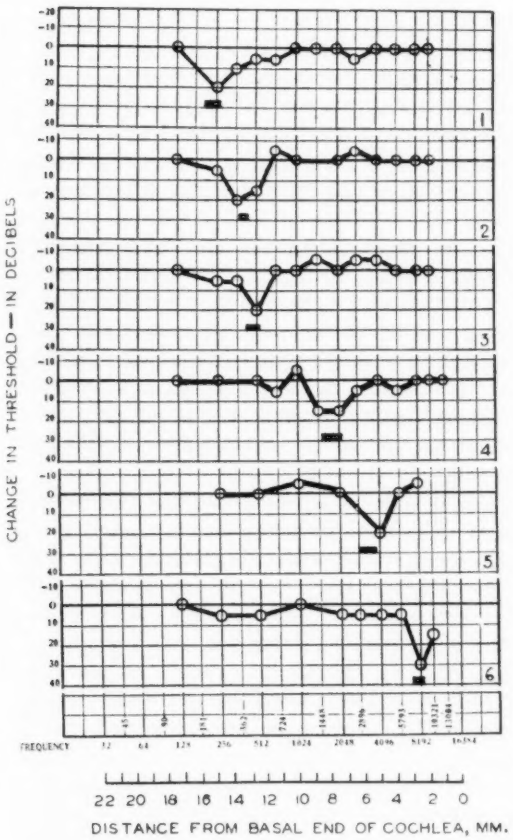

Fig. 3

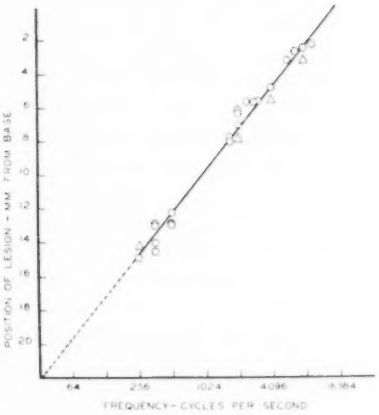

Fig. 4

in the mid-modiolar section for the basal turn by means of an ocular micrometer. Distances a and b are determined by counting the number of sections from points A to B and from A to C, and multiplying each number by the thickness of the sections. The correction factor for the vertical axis of the graph is $\frac{2c}{a+b}$. Since the vestibular part of the organ of Corti is not in the same plane as the

other turns, distances in this region were determined from chords measured with an ocular micrometer.

RESULTS. The average length of the organ of Corti in 30 cat cochleae, as measured at the junction of the inner and outer pillar cells, is 21.5 mm. The cat's cochlea has approximately three turns, but a part of each turn, namely, the region next to the brain, is inaccessible to operation. The accessible regions, in the respective turns, extend between the following levels, measured in millimeters from the extreme basal end of the organ of Corti: basal, 2.0 to 8.0; middle, 12.0 to 15.5; apical, 17.5 to 19.0.

Single small lesions. The results of six typical experiments with small lesions at representative parts of the cochlea are shown in figure 3. In these charts, the changes in threshold of response (in decibels) following the production of the lesion are plotted against the frequency of the stimulating tone. The short black bar in each case gives the position and length of the lesion, in millimeters from the basal end (lower scale of chart).

In the experiment represented by the uppermost chart, the lesion was 0.7 mm. long (from the 14.5 to the 15.2 mm. levels, measured from the basal end); and the maximum impairment of response was for the frequency of 256 cycles. In the experiment shown in the lowermost chart, the lesion extended from the 2.3 to the 3.0 mm. levels; and in this case the greatest impairment of response was for 8192 cycles. Lesions at intermediate levels, as shown in charts 2 to 5 of figure 3, caused intermediate parts of the hearing range to be affected.

The results of 21 experiments similar to those shown in figure 3 are summarized by the circles in figure 4. In this chart, the tonal region for which there was maximum impairment of response is plotted against the midpoint of the lesion, expressed in mm. from the basal end of the organ of Corti. For the frequencies from 256 to 10321, the points fall closely along a straight line, and the distances between the loci for successive octave-interval tones are the same, about 2.5 mm. per octave. The position thus indicated for the frequency of 1024 cycles cannot be experimentally determined, because it is inaccessible to operation. For the frequencies below 256 cycles per second, conclusive evidence of localization has not been obtained. However, the distance from the apical end of the organ of Corti to the position indicated for 256 is sufficient for a spacing of octaves from 32 to 256 at the same intervals as for frequencies above 256. This fact is indicated in figure 4 by the extrapolated line (dashes). No points are shown for the frequencies below 256 cycles because small lesions of the apical region did not give discrete impairments for single frequencies. Usually, small lesions of the apical region caused no discernible impairment of potentials for any tone, and with larger lesions the impairment was for more than one tone. Destruction of the entire apical end of the cochlea, down to the region indicated for 256 cycles, caused only slight to moderate impairment of response for any of the low tones.

One possible explanation of our inability to demonstrate impairment of threshold responses of cochlear potentials after apical lesions is that for very low frequencies there may be a marked spread from the region of optimal response at intensities so close to threshold that the impairments are too small to be measured

by our method. A second possible explanation is that the position of the electrode was not favorable for picking up potentials generated in the apical end of the cochlea. This explanation is favored by the results of a few experiment on guinea pigs in which the electrode was placed near the apical end of the cochlea.

Dworkin (2), by the conditioned-reflex method, found the upper limit of hearing of the cat to be about 30,000 cycles, and Wever (3) has obtained cochlear responses for this frequency. Extrapolation of our data to the position corresponding to the extreme basal end of the cochlea would indicate an upper limit of about 16,000. We have not tested for frequencies above 10,321 cycles, and have no data with respect to localization for these extremely high-pitched, unmusical frequencies; however, we do not believe the discrepancy significant.

For man Crowe, Guild and Polvogt (4) found, by correlation of hearing tests and histologic preparations, that the region of greatest importance for the reception of 8192 cycles is about 5 mm. from the basal end of the cochlea and the corresponding regions for 4096 and 2048 cycles 8 to 9 mm. and 11 to 13 mm., respectively. The only lower frequency for which similar data have been reported is 256 cycles, for the hearing of which Oda (5) estimated the region 21.5 mm. from the basal end to be most important. The total length of the human cochlea is about 31.5 mm. (Hardy, 6) as compared to 21.5 mm. in the cat, but the hearing range of both contains approximately the same number of octaves. The distances from the basal end assigned by the above authors for the frequencies 8192, 4096, 2048 and 256 cycles were multiplied by 2/3 and plotted on figure 4 (triangles). It is evident from this figure that if allowance is made for the difference in length of the cochleae of cat and man, tonal localization is the same for cochlear potentials in the cat as for hearing in man. This can only mean that cochlear potentials are closely related to actual hearing.

Our results, however, are not in agreement with the plotting of tonal localization for man by Stevens, Davis and Lurie (7) on the basis of the integration of difference limens, since these authors found the frequency distribution curve to be sigmoid, with the octaves below 500 cycles grouped near the apex.

The studies of localization in the guinea pig reported by Stevens, Davis and Lurie and by Culler (8) also show grouping of low tones in the apical turn and clearly are not in agreement with our findings for the cat.

Using guinea pigs, Held and Kleinknecht (9) abolished the Preyer reflex to some of the high notes of a Galton whistle by drilling small holes over the spiral ligament of the basal turn. The location of their lesions is not sufficiently described to permit making a close comparison with our observations, but the localization indicated for the guinea pig by Sato (10), who supplemented the method of Held and Kleinknecht by graphic reconstructions, is in good agreement with our findings in the cat for the three tonal regions he reported, namely, 240–288, 1000–1500, and 4000–4600 cycles per second.

The six graphs of figure 3 show that it is possible, by detaching the spiral ligament, to produce small lesions of the organ of Corti which result in sharply localized impairment of response for frequencies from 256 upwards, and that there is consistent correspondence between the position of the lesion and the

part of the tonal range affected. These experiments with single small lesions indicate that each region of the cochlea responds optimally to a specific frequency. It should be noted, however, that in none of the experiments was there complete loss of response for any tone, even though histological examination shows that the organ of Corti was completely destroyed in the region of the detachment. This indicates that the cochlea does not behave as a series of sharply tuned resonators, but rather that, as a sound increases in intensity, regions of the organ of Corti other than the one of optimal response also become stimulated.

The effect of the size of the lesion. Chart 1 of figure 5 shows impairments of response greater, both in amount of maximum impairment and in range of tones affected, than would be expected from the difference between the length of the lesion in this experiment and in those recorded in the charts of figure 3. Other experiments with lesions approximately 2 mm. long gave similar results. Furthermore, similar effects were obtained when a small lesion was first made and later extended in length. In the experiment recorded in chart 2 of figure 5, the small lesion (indicated by the solid part of the bar) caused the impairments of response shown by the solid line; the extension of the lesion (cross-hatched part of the bar) resulted in the impairments of responses shown by the broken line.

These experiments with larger lesions add support to the view that regions of the organ of Corti rather distant from that of optimal response for threshold intensities are of considerable importance when loud tones are used.

Multiple lesions. Experiments were made with two lesions, separated by a region of normal organ of Corti, to get some indication of the extent of the area that responds to a tone which is above threshold intensity. When both lesions were in the basal turn (in the region accessible to operation, or from the 2 to the 8 mm. levels), the impairments caused by the first lesion were increased by the second lesion. An example is shown in the uppermost chart of figure 6. In this experiment, detachment of the spiral ligament in the region from 3.3 to 3.7 mm. from the basal end (solid bar) resulted in raising the thresholds about 10 decibels for the frequencies from 5793 to 10321 cycles, inclusive. After a second detachment (cross-hatched bar) was made in the region from 6.6 to 7.7 mm. from the basal end, there was *a*, an impairment of response for the frequency (2048 cycles) corresponding to the region; *b*, an impairment for the frequency (4096 cycles) corresponding to an area between the two detachments, and *c*, an additional impairment of 20 decibels for the frequencies affected by the first lesion.

In experiments with the second lesion at about the 14 mm. level (middle turn), there was no additional impairment of response for the frequencies affected by a basal-turn lesion, indicating that the response for high frequencies (above 2048 cycles) does not extend as far as the 14 mm. level even when the intensity is 30 decibels above normal threshold. The results of one of these experiments are shown in the lowermost chart of figure 6. Detachment of the spiral ligament in the region from 2.7 to 3.8 mm. from the basal end (solid bar) raised the thresholds for 4096 and 8192 cycles 30 decibels. A second detachment, in the region

from 13.5 to 14.3 mm. (cross-hatched bar), caused a rather general impairment of response for all frequencies from 256 down, but did not cause additional impairment for the high tones.

When the first lesion made is in a region of optimal response for a low tone, making a second lesion in a region optimal for some high tone, such as 4096 or 8192, not only causes a loss for high tones but also increases the impairment caused by the first lesion. The middle chart of figure 6 shows the results of one of these experiments. The first lesion (solid bar) raised the thresholds of response for 128 and 256 cycles about 10 decibels. The second lesion (cross-hatched part of bar in basal region) caused not only an impairment of 20

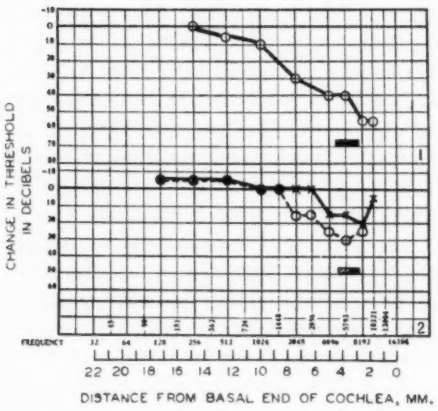

Fig. 5

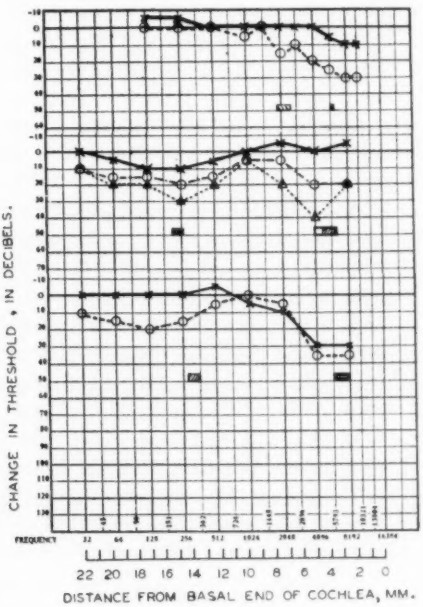

Fig. 6

decibels for 4096 and 8192 cycles, but also an additional loss for all frequencies below 512 cycles. Extension of the basal-turn lesion a half-millimeter toward the apex (open part of the bar) increased the impairment of responses for all frequencies affected by both of the previous lesions.

In the experiments with multiple lesions, it was not possible to determine the limits of the areas that respond to high tones at intensities above normal threshold because the critical levels appear to lie in the regions inaccessible to operation. These experiments do demonstrate, however, that the apical limit of the area of response to any high tone (above 2048 cycles) lies between the 8 and the 14 mm. levels, and that the areas of response for low tones (256 cycles down) extend into the basal turn at least as far as the 5 mm. level. This evidence of

sharper localization for high than for low tones is in agreement with the clinical observation that marked differences in acuity of hearing for high tones only an octave apart frequently occur, but similar differences never are present for low tones.

Discussion. In any study that utilizes the Wever-Bray phenomenon to measure the reactions of the inner ear to sound, the validity of deductions with respect to actual hearing depends on whether or not cochlear potentials originate in the hair cells of the organ of Corti.

The theories that assign the origin of cochlear response to either Reissner's or the basilar membrane, whether as streaming potentials (Eyster, Bast and Krasno, 11) or as concentration potentials (Hallpike and Rawdon-Smith, 12), no longer seem tenable, because such theories cannot possibly explain the observations of the present series of experiments or the results of the experiments in which Walzl (13) replaced perilymph with solutions of widely different ionic concentrations.

The best proof that cochlear potentials do originate in the hair cells of the organ of Corti and are, therefore, an expression of their functional activity is the fact that even very loud sounds fail to generate cochlear potentials in the ears of animals known to be deaf because of malformations of the organ of Corti (albino cats, Howe and Guild, 14; Howe, 15; waltzing guinea pigs, Davis et al., 16; Dalmatian dogs, Hughson et al., 17). The essential lesion in the ears of each of these animals is absence of the hair cells. The close correspondence between tonal localization for cochlear potentials, found in the present series of experiments, and that for hearing in man, is confirmatory evidence that cochlear potentials do arise in the hair cells of the organ of Corti and that they are "indicative of the essential functioning of the receptor" (Wever, 18, p. 50).

Several of the numerous theories of hearing include the assumptions that there are cochlear regions of optimal response for each tone, with the highest tones at the basal end, and that when the intensity of a sound is increased there is spread of the extent of the area stimulated. The experiments reported in this paper do not exclude the possibility that any one of these theories may be correct.

In the past, a serious objection to all "place" theories of hearing has been the lack of evidence to show that any given region of the cochlea is represented in the cortex by a corresponding localized area. Certainly, any form of peripheral analysis of sound is meaningless without at least an equal degree of localization at the level of the cerebral cortex. Two recent reports indicate that an orderly arrangement of connections from the several regions of the cochlea to the higher centers of the auditory system does exist. Ades (19) found that cortical response to a "click" sound was abolished in restricted parts of the auditory area of the cat's cortex by small lesions of the medial geniculate body. Woolsey and Walzl (20) stimulated small groups of nerve fibers in the operatively exposed edge of the osseous spiral lamina of the cat cochlea and obtained cortical responses (positive potentials) over restricted parts of the auditory area, different for each region of the cochlea stimulated. The cortical points of maximum

response showed a systematic arrangement, from fibers at one end of the cochlea to those at the other.

Some form of resonance or "place" theory seems best to fit the facts presented by previous clinical and experimental observations, by the present series of experiments, and by the new data with respect to the organization of the central nervous system. Sufficient information is not yet available to permit statements as to the extent of the area of the organ of Corti stimulated by a tone above threshold intensity, but it seems definite that the areas are smaller for high than for low tones and that the regions of optimal response are spaced at approximately equal distances for octave intervals of pitch.

SUMMARY

The effects of localized lesions of the cat's organ of Corti on the thresholds of cochlear potentials were studied. Histologic examination showed that the lesions were restricted to the organ of Corti, and that both Reissner's and the basilar membranes had remained intact. Graphic reconstructions were made to determine the exact location of each lesion.

Single small lesions resulted in moderate impairment of response limited to a small part of the frequency range. Larger lesions caused a greater degree of impairment and affected a wider part of the tonal range than did small lesions. Multiple lesions were used to study the extent of the area of response to tones above threshold intensity.

The following conclusions were drawn from these experiments:

1. That near threshold the response to any frequency is localized to a small part of the organ of Corti (high tones being toward the base), but that with increase in intensity there is spread of the response to adjacent areas, the spread being greater for low than for high tones.

2. That the regions of optimal response for successive octaves are spaced at equal distances along the organ of Corti.

3. That since there is close correspondence between localization for cochlear potentials in the cat and for hearing in man, cochlear potentials originate in the hair cells of the organ of Corti.

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RENAL BLOOD FLOW IN EXPERIMENTAL RENAL HYPERTENSION[1]

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Experimental renal hypertension due to renal arterial compression (Goldblatt, Lynch, Hanzal and Summerville, 1934) as well as the renal hypertension due to fibro-collagenous perinephritis (Page, 1939) have been widely thought of as responses to renal ischemia, in which the increase of arterial pressure might in some measure compensate for a decrease of excretory capacity. The demonstration of unimpaired urea clearance during renal hypertension established the maintenance of at least partial functional excretory integrity in this condition (Goldblatt, Lynch, Hanzal and Summerville, 1934). Subsequent observations of other renal clearances (phenol red, inulin and creatinine) (Corcoran and Page, 1938; (Alpert and Thomas, 1940) confirmed the supposition that glomerular filtration rate was not necessarily impaired in experimental renal hypertension and indicated the probability that renal blood flow to peritubular areas was not affected. Nevertheless, observations of renal blood flow in dogs made experimentally hypertensive by renal arterial compression have been reported, and in these renal blood flow decreased from 18 to 63 per cent (mean, 40 per cent) during the persistence of hypertension (Levy, Light and Blalock, 1939), while numerous observations have established the common occurrence of histological evidences of renal ischemia in experimental renal hypertension (Goldblatt, 1938).

A decision as to the rôle of renal ischemia in a genesis of experimental renal hypertension can only be reached by studies in which the attempt is to excite hypertension with a minimal degree of interference with renal circulation, since tight compression of the renal artery may result in both renal ischemia and hypertension, but without a necessary causal relationship between the two effects. The present communication reports results obtained from determination of renal clearances, tubular excretory capacities and total renal blood flows in dogs in which renal hypertension was purposely excited with the least possible interference with renal circulation.

MATERIAL AND METHODS. The experimental dogs were trained, mature bitches. Uninephrectomy was done in most instances several months before the first observations of renal fuction. There were three series of observations

[1] The data here reported were presented to the 50th (1938) and 53rd (1941) annual meetings of the American Physiological Society.

made. In the first, the determinations included phenol red, inulin, creatinine, or urea clearances at intervals before and after the development of hypertension due to compression of the renal artery (Goldblatt, Lynch, Hanzal and Summerville, 1934). Subsequent observations in one of these included determinations of diodrast clearance and maximum tubular excretory capacity (T_{m_D}) for diodrast and reabsorptive capacity (T_{m_G}) of glucose by methods modified from those of Smith, Goldring and Chasis (1938). The second series consisted of uninephrectomized dogs with single kidneys explanted subcutaneously by the technique of Page and Corcoran (1940), in which determinations of absolute renal blood flow calculated from phenol red and inulin clearances and extraction percentages were made at intervals before and after the onset of hypertension due to clamping the renal artery. Most of the chemical methods used in this study have been described elsewhere (Corcoran and Page, 1939).

TABLE 1

Phenol red and inulin plasma clearances in renal hypertension due to arterial compression

Renal clearances and mm. Hg blood pressure in dogs 1 and 2 before and after renal arterial compression. Day = day from first observation and hr. = hours after application or adjustment of clamp. Abbreviations: P.R., phenol red; I., inulin; U., urea; C., clearance (cc. per sq. m. per min.); B.P., blood pressure mm. Hg.

DOG NO.	DAY	RENAL P.R.C.	PLASMA I.C.	CLEARANCE U.C.	CLEARANCE P.R.C./I.C.	B.P.
						mm. Hg
	Left nephrectomy					
1	95	129	90	62	1.44	144/84
	98	130	90	55	1.44	142/84
	99	127	73	52	1.72	140/84
	104	108	82	52	1.32	158/98
	Right clamp. Local anesthesia					
	(1 hr.)	110	84	38	1.32	194/124
	106	120	75	57	1.61	148/94
	110	116	76	46	1.53	158/104
	Clamp tightened 1 turn. Local anesthesia					
	(1 hr.)	36	15.7	3.4	2.30	212/146
	112	86	97	40	0.89	248/168
	Clamp removed. Local anesthesia					
	(1 hr.)	119	93	44	1.28	220/158
	117	121	89	58	1.36	180/110
	118	94	71	34	1.28	156/96
	Clamp reapplied. Local anesthesia					
	133	106	88	41	1.20	162/96
	173	121	76	51	1.59	230/150
	185	123	87	47	1.41	210/132
2	62	84	53	25	1.58	132/84
	67	101	57	29	1.77	140/86
	71	85	46	27	1.85	130/82
	79	Right clamp. Local anesthesia				
	(2 hr.)	31	33	2.3	0.94	228/146
	(7 hr.)	65	37	10.3	1.75	198/124
	80	Loosen clamp ½ turn. Ether anesthesia				
	81	110	63	34	1.75	192/126
	85	83	44	15	1.88	206/138
	89	79	54	22	1.46	208/132
	95	93	50	18	1.86	206/134
	101	93	50	27	1.86	206/122

The values of renal blood flow and extraction presented are average of at least three successive determinations made on the same day. In some cases in both series, the degree of renal arterial compression was varied by opening the wound and suitably adjusting the clamps. A third group of observations were made in dogs in which hypertension due to perinephritis was induced by bilateral perirenal application of silk. The determinations made in these include diodrast and inulin clearances and T_{m_D}. Diodrast determinations were made by a modification of the method of White and Rolf (1940). The values of renal blood flow, clearance, and tubular capacity are presented in cubic centimeters or milligrams per square meter of body surface per minute.

Blood pressure (B.P.) in the first series was determined by auscultation over the dorsalis pedis artery (Allen, 1923) while, in subsequent series, it was measured by direct femoral arterial puncture.

Note should be made of our unfortunate experience in the application of metal clamps to the renal arteries of dogs with single explanted kidneys. In several instances, death, due to renal arterial thrombosis, followed soon after clamping, apparently because of the dog's lying on the insensitive explanted kidney, and compressing the renal artery against the rigid clamp. The incidence of such accidents was greatly decreased by the postoperative application of a rigid shield over the explanted kidney.

Histological examination of tissues from those dogs which died was done by Dr. Irving Graef, Department of Pathology, New York University, to whose kindness we are indebted for summarized reports.

RESULTS. *1. Clearance determinations.* Observations of phenol red, inulin and urea clearances in two dogs are tabulated (table 1). In two dogs (nos. 3 and 4) only creatinine and urea clearances were determined. Determinations on the first, second and fourth days after clamping showed no significant changes during increases of arterial pressure to levels of 204/125 and 170/110 mm. Hg. Observations in another dog (no. 5) whose surface area was 0.570 sq. m., are of particular interest, since they extend over nearly 3 years. Briefly, there were no significant variations of creatinine and urea clearances during 2 months after clamping. The B.P. varied from 178/98 to 228/190 mm. Hg during this time (control value 140/83). The blood pressure level fell to average about 168/110 mm. Hg during the next six months, while phenol red and inulin clearance values were respectively 121 and 72 cc. per square meter per minute. The clamp was then tightened and, on the following day, B.P. was 224/154 and phenol red and inulin clearances respectively 80 and 91 cc. Clearance determinations at intervals during the following 13 months yielded mean values of 125 (phenol red) and 71 cc. (inulin) with mean blood pressure 220/145 mm. Hg. Determinations of diodrast and inulin clearances, tubular secretory capacity (T_{m_D}) and tubular reabsorptive capacity (T_{m_G}) were made at intervals during the following 8 months. The mean values and range of these nine determinations, corrected to one square meter of body surface were as follows: diodrast clearance, 275 cc., inulin clearance 92 cc., T_{m_D} 15.4 mgm., T_{m_G} 293 mgm. per minute. Blood pres-

sure during this time averaged 155 mm. Hg and ranged from 160 to 202 mm. Hg. The last determinations of T_{m_D} (15.3 mgm.) and T_{m_G} (271 mgm.) were made at a time when retinal hemorrhages and partial retinal detachment boded the onset of the "malignant syndrome". Histological study of the kidney, which weighed 80 grams, showed one tiny scar of the ischemic type and rare hyaline glomeruli. There was some increased prominence of the "polkissen" in the renal cortex.

To summarize, hypertension due to compression of the renal artery was observed in dogs without constant or persistent reduction of phenol red, inulin, creatinine or urea clearances, although it was sometimes more severe during phases of reduction of clearance. The values of T_{m_D} and T_{m_G} and the size of the kidney (dog 5) do not indicate failure of tubular function nor atrophy of tissue with reference to normal values (White and Heinbecker, 1940; Shannon, Farber and Troast, 1941).

2. Observations of absolute renal blood flow. Data representative of the group of 18 are presented from observations made in 6 dogs, in some of which the studies were extended over several months.

a. Short periods of observation: Hypertension was obtained in one instance (dog 6) without a significant change of renal blood flow; varying degrees of renal ischemia developed in others (e.g., dog 7).

b. Long periods of observation: Hypertension was observed over long periods in 4 subjects without definite relationship being found between the levels of mean arterial pressure and renal blood flow. Data from these experiments are presented in summarized protocols (dogs 8, 9, 10 and 11) and in figures 1 and 2.

3. Renal function in hypertension due to perinephritis. Observations made in one subject are summarized in table 2, in which it is shown that hypertension developed without a decrease of plasma diodrast clearance (effective renal plasma flow) or an initial failure of maximal tubular secretory capacity (T_{m_D}). The late fall of T_{m_D} may represent tissue loss due to infiltration with scar. There is, however, no evidence of ischemia of the residual tubular mass, i.e., no depression in the ratio diodrast clearance/T_{m_D}.

Discussion. *1. Clearance studies.* The plasma clearances of inulin and creatinine in the dog equal the rate of glomerular filtration, while phenol red clearance depends largely upon tubular secretion and is equivalent to roughly one-half of renal plasma flow with which it varies directly. The formulation of this relationship has been previously described (Corcoran and Page, 1939). Diodrast plasma clearance depends largely upon tubular secretion and is probably equivalent to nearly all the renal plasma flow to excretory tissues (Corcoran, Smith and Page, 1941). It is therefore termed "effective renal plasma flow" (Smith, Goldring and Chasis, 1938). The maintenance of normal levels of these clearances in dogs during renal hypertension is evidence against the causal existence of renal ischemia in this condition, while the adequacy of filtration rate, of tubular secretory and reabsorptive capacities indicate that the hypertension is not associated with excretory impairment. It is, however, evident

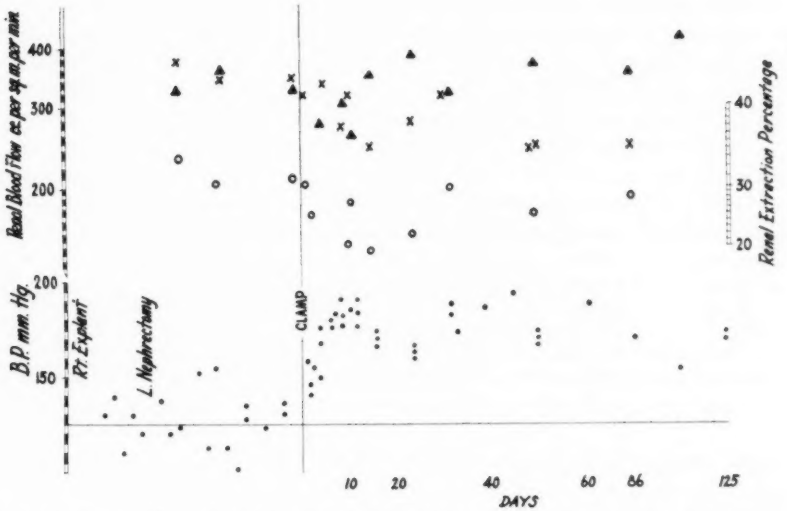

Fig. 1. Observations of total renal blood flow (▲), phenol red, *X*, and inulin, *O*, extraction percentages and femoral arterial pressure in dog 10 before and after renal arterial compression.

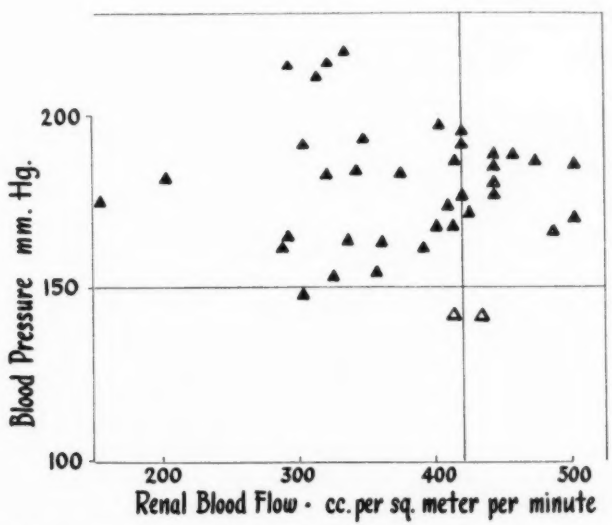

Fig. 2. Observations on the relation of total renal blood flow and mean arterial pressure, before (△) and after (▲) the production of hypertension by renal arterial compression in dog 11.

that exact knowledge of the relation of renal ischemia to renal hypertension can best be attained by measurements of total renal blood flow.

2. Renal blood flow and extraction percentage. The measurements of renal blood flow were made from determinations of phenol red and inulin clearances and extraction percentages, and therefore represent total renal blood flow without reference to the tissues perfused. The data presented from dogs 6 and 10

TABLE 2

Diodrast and inulin plasma clearances and Tm_D during hypertension due to perinephritis

Renal clearance, tubular excretory capacity (diodrast) and blood pressure before and after production of hypertension by application of silk to both kidneys. Dog 12. Surface area 0.61 sq. m. Abbreviations: D., plasma diodrast; I., inulin; F.F., filtration fraction (IC/DC); C., clearance in cubic centimeters per square meter per minute; Tm_D, tubular secretory capacity for diodrast in milligrams per square meter per minute; V.C., vasoconstrictor content of peripheral blood (Page, 1940) by comparison with blood of normotensive dogs.

	D.C.	I.C.	F.F.	Tm_D	D.C./Tm_D	B.P.	V.C.
						mm. Hg	
Control mean	228	107	35.2	27.0	10.7	142	1.0
Control maximum	321	129	41	28.1	11.8	166	
Control minimum	259	81	26	25.9	9.6	114	
Observation no.	16	16	16	4		22	
PERINEPHRITIS							
day							
4	205	54	26			130	
7	241	86	36			150	3.3
10	308	116	38	26.8	11.5	167	1.2
15	384	111	39			161	
21	269	112	41	27.2	9.85	180	
24	264	99	37			164	
34	280	106	38	25	11.2	175	
37	333	128	39			187	1.3
42	338	120	36			180	
40	315	121	38	28.8	10.9	181	20
54	325	105	33	27	12	180	8.2
57	332	128	39			187	19
72	248	95	32	22.5	11	189	9.7
78	308	109	35.4	22.0	14	193	8.2
Hypertension							
Days 21–78 mean	305	112	36 8			182	
Days 21–78 maximum	338	128	41	28 8	12 0	193	19.0
Days 21–78 minimum	264	94.5	31.7	22 0	9 85	164	1.2

illustrate the development and persistence of renal hypertension without significant persistent renal ischemia while the absence of correlation of renal blood flow and arterial pressure is established in the other reported observations.

The renal extraction of inulin was of particular interest, since the so-called humoral mediators of hypertension, renin and angiotonin, tend to decrease renal blood flow and increase inulin extraction, apparently by causing efferent arteriolar constriction (Corcoran and Page, 1939, 1940a, b). Efferent arteriolar

constriction is characteristic of the renal circulation in hypertension of human beings (Goldring, Ranges, Chasis and Smith, 1938). It would be expected that participation of these mediators of hypertension in renal hypertension in dogs would be expressed in an increased inulin extraction and a secondary decrease of renal blood flow, at least during periods of severe hypertension, and this supposition is confirmed in some of the observations on dogs 6 and 11. However, compression or obstruction of the renal artery may, by reducing intraglomerular pressure, decrease inulin extraction as occurred in dogs 7 and 8 and in unreported observations. Such low values of inulin extraction probably represent considerable reductions of effective intra-renal arterial pressure, since they were obtained during levels of renal blood flow at least 30 per cent below control levels and, in some instances, in the absence of hypertension. Since the exact level of inulin extraction percentage after renal arterial compression is a product of the change in renal arterial pressure and of variations in afferent and efferent arteriolar tone, evidence of efferent constriction from inulin extraction alone cannot always be obtained.

3. Hypertension due to perinephritis. The data of table 2 establish that hypertension due to the production of fibro-collagenous perinephritis after application of silk crepe to both kidneys may persist without changes of effective renal plasma flow, or, initially of tubular secretory capacity (T_{m_D}). The increase of the inulin/diodrast clearance ratio (the equivalent of inulin extraction ratio) during the development of hypertension presumably reflects increased intraglomerular pressure, which may result from efferent arteriolar constriction. The appearance of the increased amounts of an angiotonin-like vasoconstrictor (Page, 1940) also speaks for the participation of the renin-angiotonin pressor system in the course of this hypertension.

4. Renal blood flow in experimental hypertension. The mechanism by which compression of the renal artery by a metal clamp or of the renal parenchyma in the scar of perinephritis may lead to the development of hypertension in the absence of persistent renal ischemia has been reviewed elsewhere and confirmatory observations cited (Corcoran and Page, 1941). The topic need not therefore here be developed in detail. Briefly, the view is proposed that the rate of renal blood flow in experimental renal hypertension depends upon a balance struck between increased arterial pressure and increased renal resistance, due either to primary arterial compression or compression of the renal parenchyma or, secondarily, to renal vasoconstriction induced by the unopposed activity of the humoral mediators of renal hypertension. Both the factors which produce the hypertension and the renal vasoconstriction which may result from it will, when sufficiently intense, reduce renal blood flow in spite of increased arterial pressure. But, in the hypertensive animal, if the ratio of arterial pressure to renal resistance is that which obtains in normal animals, renal blood flow may remain within normal limits. The disparity between the intense renal ischemic effect of renin and angiotonin in normotensives and their presumed action in hypertension may depend upon the inability of the normotensive to maintain the level of cardiac output during a sudden increase of peripheral resistance.

In the absence of renal ischemia the decrease of pulse pressure or some more subtle variation of pulse wave, which follows clamping the renal artery or which may occur in a kidney rigidly held in the firm scar of fibrocollagenous perinephritis, remains as a possible cause of the intrarenal hemodynamic changes which lead to hypertension. This view is supported by the liberation of renin which follows perfusion with blood of the isolated dog's kidney under reduced pulse pressure with constant mean arterial pressure and renal blood flow (Kohlstaedt and Page, 1940). Observations which suggest hypertrophy of the "polkissen" (Goormatigh and Grimson, 1939) and which have led to the supposition that these bodies may be concerned in the genesis of renal hypertension may be cited in this connection. Evidence of such changes in the "polkissen" were observed by Graef in our dogs 6, 7, 9 and 11. The pre-glomerular position of the "polkissen" is such that they are exposed to variations of pulse pressure in the afferent arterioles.

The possibility that renal hypertension is due rather to a redistribution of blood flow within the kidney, as by pre-glomerular shunting, with resultant ischemia of certain areas, is not excluded by the observations of renal blood flow, but seems to be unlikely from the observations of clearance and excretory capacity, since both depend upon the blood reaching glomerular and tubular areas.

SUMMARY AND CONCLUSIONS

1. Hypertension due to renal arterial compression or compression of the renal parenchyma in perinephric scar may occur without constant or persistent changes in the renal clearances of diodrast, phenol red, inulin or urea and without significant abnormalities of tubular excretory or reabsorptive capacity and, therefore, probably in the absence of ischemia of excretory renal tissue.

2. Measurements of total renal blood flow from the clearances and extraction percentages of phenol red and inulin indicate no correlation of mean arterial pressure with the rate of renal blood flow, and establish the persistence of hypertension in the absence of renal ischemia. Evidence of renal vasoconstriction, predominantly of the efferent arterioles, was obtained from the levels of inulin extraction and renal blood flow in some dogs during phases of more severe hypertension and in association with secondary renal ischemia. In one dog in which hypertension was the result of perinephritis, intraglomerular filtration pressure was increased in the absence of changes in "effective renal blood flow". Ischemia due primarily to compression or occlusion of the renal artery was usually associated with decreased renal extraction of inulin, due apparently to decreased filtration pressure.

3. The persistence of experimental renal hypertension in the absence of renal ischemia is consistent with the view that intra-renal reduction of pulse pressure rather than ischemia may be the effective cause of experimental renal hypertension.

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Protocols. Dog 6; 0.638 sq. m. Right nephrectomy, left renal explant. During observations in the control period (6 mos.), blood pressure varied from 98 to 138 and averaged 122 mm. Hg, while renal blood flow averaged 430 cc. and varied from 380 to 460 cc. in 4 observations. Two days after clamping the renal artery, blood pressure was 157 mm. Hg and renal blood flow on the 5th day was 410 cc. (mean of 3 periods). In both these postoperative determinations of renal blood flow I. Ep. (inulin extraction percentage) remained nearly equal to the mean control level of 29.4 per cent (range 26.6–33.8). Seven days postoperatively renal blood flow was 349 cc. and blood pressure was 178 mm. Hg, while I. Ep had increased to 37 per cent. Renal arterial thrombosis developed on the evening of the 7th day and, on the 9th day, blood pressure had fallen to 130 mm. Hg and blood urea nitrogen was 123 mgm. per 100 cc. Histological examination showed that the renal parenchyma was comparatively well preserved, although there were foci of tubular degeneration in cortex and medulla. There was necrotizing arteriolitis in the submucosa of the small intestine and focal necrosis with calcification in the myocardium. The renal artery showed antemortem thrombosis with organization and canalization. The renal vein was collapsed by hemorrhage into its sheath. *Summary:* Hypertension following clamping without reduction of renal blood flow, although renal ischemia with increased I. Ep. subsequently developed. Fall of blood pressure and death in uremia was the result of renal arterial thrombosis with obstruction of the renal vein.

Dog 7; 0.946 sq. m. Left explant and right nephrectomy. Renal blood flow in control periods was 277 cc. (range 246–308—3 observations) and I. Ep. was 32.9 per cent (range 29–39 per cent). Blood pressure during this time was 117 mm. Hg mean value (range 105–131). No increase of blood pressure occurred during the two days after clamping during which time renal blood flow was 252 cc. and I. Ep. 32 per cent on the first day, and renal blood flow 290 cc. and I. Ep. 30.5 on the 2nd day. The clamp was therefore tightened one full turn of the screw and blood pressure increased to 153 mm. Hg on the following day, while renal blood flow decreased to 176 cc. and I. Ep. to 22.7 per cent. The clamp was loosened one-half turn on the next day, at a time when blood pressure was 164 mm. Hg, renal blood flow 114 cc. and I. Ep. 24.4 per cent. Blood pressure returned to 123 mm. Hg on the day after loosening the clamp and blood urea nitrogen fell from 86 to 35 mgm. per 100 cc. Renal blood flow at this time was 208 cc. and I. Ep. 29 per cent. Blood pressure increased during the next 3 days to a mean of 154 mm. Hg and, on the 4th day after loosening the clamp, was 162 mm. Hg, renal blood flow was 121 cc. and I. Ep. 15.3 per cent. Death occurred from malignant hypertension 7 days after the clamp had been loos-

ened. Histological examination showed necrotizing arteriolitis in the pancreas, adrenal capsule, stomach and jejunum. The renal parenchyma was well preserved in some sections, while others showed areas of medullary calcification. The "polkissen" were prominent particularly in the outermost glomeruli. Many exhibited pyknosis of nuclei of the constituent cells. *Summary:* Renal ischemia and decreased I. Ep. with return of blood pressure and renal changes toward normal after loosening the clamp. Recurrence of hypertension, renal ischemia and decreased I. Ep. due to renal arterial thrombosis. Malignant hypertension without severe increase of arterial pressure.

Dog 8; 0.539 sq. m. Left renal explant, right nephrectomy. Control renal blood flow 331 cc. (12 observations, range 299–404 cc.) and I. Ep. 25.3 mean (range 18.9–35 per cent). Blood pressure during this time averaged 137 mm. Hg (range 101–158 in 52 observations). On the second day after application of a clamp to the renal artery, blood pressure was 162 mm. Hg and renal blood flow 259 cc. with I. Ep. 24 per cent, and, on the 10th day, blood pressure was 144 mm. Hg and renal blood flow 224 cc. with I. Ep. 28.0 per cent. Mean blood pressure during the two weeks postoperatively was 148 mm. Hg. The clamp was therefore tightened one-quarter turn. Blood pressure did not increase, but renal blood flow on the 2nd day after tightening was 293 cc. with I. Ep. 24.3 per cent. The clamp was again tightened one-quarter turn and, during the following week, blood pressure increased to values ranging from 160 to 186 mm. Hg with renal blood flow averaging 284 cc. and I. Ep. 28.1 per cent. Two weeks after that the blood pressure was 143 mm. Hg, so that the clamp was again tightened. Blood pressure during the two months after this fourth operation averaged 166 mm. Hg (range 145–173) and the mean of 5 observations of renal blood flow was 258 cc. (range 234–275). The clamp was then again tightened one-half turn. Blood pressure increased to 190 mm. Hg during the next few days and, in the ensuing 7 months, maintained an average level of 175 mm. Hg (range 144–190), while renal blood flow (8 observations) averaged 302 cc. and ranged from 245 to 366 cc. with I. Ep. averaging 27.2 per cent (range 22.9–31.7). Phases during which renal blood flow were higher than the mean, such as 267, 245, 273 cc., were associated with blood pressure values of 180, 159 and 148 mm. Hg. The I. Ep. values corresponding to the periods of higher flows were respectively 25.5, 31.8 and 33 per cent. *Summary:* Production of hypertension with renal ischemia and with a delayed increase of I. Ep. Subsequent persistence of hypertension with mean renal blood flow nearly equal to that of control observations.

Dog 9; 0.66 sq. m. Right renal explant, left nephrectomy. Control renal blood flow 266 cc. (range 242–291), I. Ep. 35.6 per cent (29.2–372) and blood pressure 115 mm. Hg (range 104–131). On the first and second days after clamping the renal artery blood pressure was 139 and 140 mm. Hg, at which time pneumonia developed and the clamp was loosened. Ten days after loosening the clamp renal blood flow was 131 cc., I. Ep. 32.1 and blood pressure 116 mm. Hg, while the values on the 21st day were respectively 158 cc., 31.6 per cent and 114 mm. Hg. The clamp was then tightened. Blood pressure on the day after tightening was 186 mm. Hg, but fell to 142 and 145 mm. Hg on the 2nd and 3rd days. Ten days later blood pressure was 142 mm. Hg, I. Ep. 35.8 per cent and renal blood flow 164 cc., when unexplained anuria developed and blood urea nitrogen content increased to 93 mgm. per 100 cc. The clamp was again loosened. During the ensuing 2 months blood pressure averaged 152 mm. Hg (range 148–165). Renal blood flow 22 days after loosening the clamp was 228 cc. and I. Ep. 26.6 per cent. During the next 6 months, renal blood flow averaged 280 cc. (range 272–313), I. Ep. 29.6 and blood pressure 158 (range 144–176 mm. Hg). The mean of 5 observations during a second 6 months period of observation yielded values of 288 cc. renal blood flow (range 227–339), I. Ep. 29.2 (22–31 per cent and blood pressure 140 mm. Hg) (range 136–168). Death was due to intestinal obstruction. Microscopic examination of the kidney revealed marked inflammatory changes in the wall of the renal artery. Tubules beneath the renal capsules were compressed at the site of a localized perirenal abscess. There were diffusely scattered scars in the cortical and subcortical areas and at the cortico-medullary junction. The "polkissen" were moderately prominent, many of them exhibiting pyknosis of nuclei of constituent cells. Histological diagnosis: low grade

chronic perinephritis with tubular compression and atrophy; focal renal fibrosis and ischemic atrophy. *Summary:* Renal ischemia sufficient to cause permanent scarring within the kidney was present initially without hypertension; hypertension of moderate degree subsequently developed in the absence of renal ischemia, possibly as a result of inflammatory changes in the renal artery.

Dog 10; 0.680 sq. m. Left renal explant, right nephrectomy. Blood pressure by auscultation averaged 148/86 mm. Hg (range 132/76–170/116) while later mean femoral arterial pressures averaged 122 mm. Hg (range 112–138) during the control period. Control renal blood flow was 335 cc. and I. Ep. 32.2 per cent. The changes which followed clamping are shown graphically in figure 1. From the 10th day after clamping renal blood flow averaged 331 cc. and I. Ep. 24.8 per cent, while arterial pressure was 174 mm. Hg (range 154–192). Death was at first attributed to colitis, since there were hemorrhages and ulceration of the ileal and colonic mucosa. Sections through one of these mucosal ulcers showed an intense leukocytic response through the muscularis, the ulcer base and submucosa. The arterioles of the submucosa and muscularis showed necrotizing arteriolitis. There was a small area of old infarction at the upper pole of the kidney, without reduction in the size of the organ. Histological examination showed the renal parenchyma fairly well preserved, although there were early autolytic changes in the tubular epithelium. The glomeruli were large and hyperemic. *Summary:* Persistence of hypertension in the absence of renal ischemia with subsequent development of questionable malignant hypertension.

Dog 11; 0.674 sq. m. Left explant, right nephrectomy. During the control period renal blood flow averaged 410 cc., I. Ep. 30.2 per cent and blood pressure 148 mm. Hg (138–160). Data obtained after clamping the renal artery extend over 15 months and are summarized in figure 2. Renal blood flow on the 3rd day after clamping was 177 cc., I. Ep. 36.4 per cent and blood pressure 172 mm. Hg. During the following 18 days renal blood flow averaged 320 cc., I. Ep. 26.8 per cent and blood pressure 190 mm. Hg. The clamp was then loosened one full turn. Renal blood flow during the 2 weeks after loosening the clamp averaged 400 cc., I. Ep. 22.2 per cent and blood pressure 176 mm. Hg (range 160–216). The clamp was then tightened one-half turn. Renal blood flow in the ensuing month averaged 401 cc., I. Ep. 25.5 per cent and blood pressure 175 mm. Hg (range 162–216). The clamp was then tightened $\frac{3}{4}$ turn. Observations during the following 6 months yielded mean values of renal blood flow of 404 cc. (range 291–508), I. Ep. 25.1 per cent (range 20.7–34.5) and blood pressure 172 mm. Hg (range 151–202). The clamp was then tightened $\frac{1}{4}$ turn. Renal blood flow averaged 401 cc. during the following three weeks, while I. Ep. was 27.8 per cent and blood pressure 173 mm. Hg (range 153–190). The clamp was again tightened $\frac{1}{4}$ turn. In the next 2 months renal blood flow was 404 cc., I. Ep. 24.8 and blood pressure 177 mm. Hg. Loss of weight and appetite with occasional vomiting during the next 2 months terminated in death from volvulus. During this period of developing cachexia, renal blood flow was 249 cc. I. Ep. 24.3 cc. and blood pressure 176 mm. Hg (range 155–185). Histological examination revealed myocardial degeneration with loss of striations and vacuolization of muscle fibers. In the kidney there was extensive scarring of the ischemic type with some tubular regeneration and replacement fibrosis, extending in wedges from the capsule to the medulla. The glomeruli were well preserved in outline, although they exhibited pericapsular fibrosis and some showed apparent hypertrophy of the "polkissen" with focal pyknosis of their nuclei. Section of the compressed renal artery revealed that there had been thrombosis and canalization. *Summary:* Persistent hypertension without consistent relationship between the levels of renal blood flow and mean arterial pressure.

THE EFFECT OF ANIMAL POISONS (RATTLESNAKE VENOM AND TRYPSIN) ON THE BLOOD HISTAMINE OF GUINEA PIGS AND RABBITS

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Feldberg and Kellaway (1) have shown that snake venoms liberate histamine from perfused isolated organs of cats, dogs, monkeys and guinea pigs. Dragstedt, Mead and Eyer (2) reported the appearance of a hypotensive substance, similar to histamine, in the blood of dogs injected with crotalin (rattlesnake venom). In previous experiments (3) it has been shown that trypsin liberates histamine from the perfused lung of the guinea pig and that the intravenous injection of trypsin into dogs (4) leads to the liberation of histamine from the liver into the circulating blood. Furthermore, trypsin has in common with the venom of certain snakes and the venom of the honeybee most of their highly interesting pharmacologic properties, such as the production of contraction and subsequent desensitization of smooth muscles; and, after the injection of appropriate doses, collapse and death of the intact animal (5). The general similarity of these effects to those seen in anaphylactic shock has been emphasized many times (5, 6).

Although the external symptoms produced by the injection of a lethal dose of crotalin or trypsin into the guinea pig closely resemble the symptoms of anaphylactic shock, a closer examination of the pathologic findings shows several discrepancies which will be discussed in this paper. The most outstanding gross feature of anaphylactic shock in the guinea pig is a pale, emphysematous lung. This feature fails to appear in most of the cases of acute poisoning by snake venom and trypsin. Crotalin, in about a third of the cases, produces definite pulmonary emphysema, but this is less pronounced than that produced by anaphylactic shock. Trypsin seems to produce still less emphysema than crotalin.

Since epinephrine is a powerful antagonist of the action of histamine on the lung of the guinea pig and since Feldberg (7) has shown recently the ability of snake venoms to liberate epinephrine in vivo and in vitro from the adrenal glands, we tried to ascertain whether the removal of the adrenals of the guinea pig would increase the degree of emphysema produced by the injection of crotalin or trypsin. The animals were adrenalectomized in the morning and in the afternoon were injected with crotalin (Crotalus atrox) or trypsin (a crystalline

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preparation). On some control animals a "sham" operation was performed, which was in all respects the same as adrenalectomy except that the glands were left in place. A third of the series of animals were given the injections of the toxins, without any previous treatment. Tables 1 and 2 show the differences of behavior between the adrenalectomized and normal animals toward the in-

TABLE 1

Effect of trypsin on adrenalectomized, normal and "sham operated" guinea pigs

NUMBER	WEIGHT	AMOUNT OF TRYPSIN INJECTED	DIED AFTER	KILLED AFTER	EMPHYSEMA	HYPEREMIA OF ABDOMINAL VISCERA
			Adrenalectomized			
	grams	*mgm.*	*minutes*	*minutes*		
1	350	20		2	±	±
2	370	15	2		+++	++
3	300	15	3		++	0
4	400	15	2		0	0
5	420	12	2		+++	±
6	530	11		10	± or +	+++
7	400	11	2		++++	±
8	620	10	10		+ or 0	++
9	350	10	15		+ or 0	+++
10	350	10	5		++	+
11	600	8	4		++	+
12	400	8		5	++	+
13	400	8	5		++	+++
14	390	8		10	++	+++
15	690	5	3		+++	±
			"Sham operated"			
16	350	25	2		0	0
17	350	25		2	0	±
18	390	20	5		±	+++
19	350	15		15	+	+++
20	400	13		10	0	+++
			Normal			
21	350	25	5		0	++
22	400	20		2	0	±
23	350	20	2		±	0
24	350	20		5	0 or ±	++
25	350	15	7		0	0 or +

jection of trypsin or crotalin. There was a definite increase in the size of the emphysematous lungs after adrenalectomy, and in respect to trypsin a definite reduction in the lethal dose was observed. Considering the trauma produced by the operation itself and the abnormal condition of the animals after adrenalectomy, the reduction of the lethal dose perhaps should be ignored. The increased emphysema in the adrenalectomized animals might be explained on

the basis of a lack of epinephrine. The emphysema was never so pronounced as that seen in guinea pigs after anaphylactic death and in most of the cases it could not explain the death of the animals injected with the toxins employed. The extreme hyperemia of the abdominal organs and the wall of the abdomen which was a common appearance in all the animals killed by trypsin, if five or ten minutes elapsed after the injection, strongly suggested the collapse of the peripheral vessels and possibly the interaction of histamine.

TABLE 2

Effect of snake venom (Crotalus atrox) on adrenalectomized and normal guinea pigs

NUMBER	WEIGHT	AMOUNT OF VENOM INJECTED	EMPHYSEMA
	Adrenalectomized		
	grams	*mgm.*	
1	300–400	10	+++
2	300–400	10	+++
3	300–400	10	++
4	320	10	+++
5	380	10	++
6	390	10	+++
7	320	6	+++
8	340	10	+++
	Normal		
9	300–400	10	++
10	300–400	10	±
11	300–400	10	±
12	350–400	10	++
13	350–400	10	++
14	350–400	10	+
15	350–400	10	++
16	350–400	10	+
17	350–400	10	+ or ++

TABLE 3

The effect of intravenous injection of crotalin (Crotalus atrox) on the blood histamine of guinea pigs

NUMBER	WEIGHT	DOSE	HISTAMINE CONTENT OF THE BLOOD		PERCENTAGE OF REDUCTION
			Before	After	
	grams	*mgm.*	*γ/cc.*	*γ/cc.*	
1	1060		0.10		
2	815	15	0.18	0.16	11
3	750	15	0.22	0.16	27
4	750	15	0.13	0.076	41
5	840	15	0.13	0.086	34
6	700	10	0.20	0.13	35
7	710	10	0.20	0.126	37
8	700	10	0.143	0.10	30
9	640	10	0.066	0.05	24
10	560	10	0.066	0.05	24
11	680	10	0.125	0.07	44
12	587	10	0.17	0.11	35
13		20	0.11	0.05	54
14		15	0.05	0.035	30
15		10	0.26	0.20	23

To check the possibility of participation of histamine in the production of the symptoms, we have tested for histamine samples of blood drawn before and after the injection of crotalin or trypsin. A reduction of the histamine content of the blood was sometimes very conspicuous after injection of crotalin and was very marked in the three cases studied after the injection of trypsin (tables 3 and 4). In every case there was a definite reduction of the histamine content of the blood. Code has shown an increase of the histamine content of the blood of guinea pigs during anaphylactic shock. Crotalin and trypsin produce a release of histamine from the lung of the guinea pig perfused in vitro but cause a decrease of the blood histamine when injected in vivo. This apparently paradoxical behavior might

be explained by the fact that these toxins when injected intravenously come first in contact with the white blood cells, which are very rich in histamine, as shown by Code (9). The release of histamine from the cells to the plasma and the consequent diffusion to the tissues would account for the reduction of the total blood histamine after the injection of crotalin or trypsin. On the contrary, in anaphylactic shock in the guinea pig the main liberation of histamine takes place when the antigen comes in contact with the tissues. A high precipitin titer in the blood seems to protect the animal against the antigen and definitely to minimize the effect of the antigen on the production of emphysema, reducing the percentage of deaths. The site of action of the injected toxins and of the antigen-antibody reaction in anaphylactic shock probably is responsible for the difference in the effects produced by them on the blood histamine and on the production of emphysema.

There is less discrepancy in the effects of these toxins and anaphylactic shock on the blood of rabbits. Rose and Weil (10) found that the injection of antigen

TABLE 4

Effect of trypsin on the blood histamine of guinea pigs

NUMBER	AMOUNT OF TRYPSIN INJECTED	HISTAMINE CONTENT	
		Before	After
	mgm.	*γ/cc.*	*γ/cc.*
1	20	0.06	0.017
2	12	0.3	0.12
3	10	0.24	0.066

TABLE 5

Effect of snake venom (Crotalus adamanteus) on the blood histamine of rabbits

NUMBER	WEIGHT	DOSE	HISTAMINE CONTENT		TIME OF DEATH
			Before	After	
	kgm.	*mgm.*	*γ/cc.*	*γ/cc.*	*minutes*
1	1.8	20	3.2	2.0	3
2	1.9	10	2.4	1.9	3
3	1.8	7	3.8	1.92	5
4	2.2	5	2.4	0.12	Died after 15 minutes
5	2.4	5	2.4	0.17	Killed after 20 minutes

into sensitized rabbits led to a reduction of the total blood histamine. In previous experiments it was shown (11) that trypsin produced the same effect. Katz (12) and Dragstedt and his associates (13) have shown that the release of histamine from cells to plasma and the resulting leukopenia explain the fate of the histamine which disappears from the blood of the rabbit. Similar facts have been shown with regard to trypsin (11). Table 5 shows a similar experiment performed with crotalin (Crotalus adamanteus). Rabbits were injected intravenously with varying amounts of the venom and the blood was drawn before and after the injection. Sometimes, when a small amount of venom was injected, a great reduction of the blood histamine was seen. The smaller reduction when larger amounts of venom were injected might be explained by a liberation of histamine from the tissues, counterbalancing in some way the disappearance of the histamine from the blood cells.

The fact that rabbits and guinea pigs behave in a similar way toward the injection of crotalin or trypsin is very suggestive and is in contrast to the fact that

the guinea pig is an exception among the rodents in respect to its great sensitivity to anaphylactic shock. The foregoing facts might contribute to an understanding of the differences in the mechanism of death of rabbits and guinea pigs in anaphylaxis, since the former die by circulatory collapse and the latter by respiratory arrest and asphyxia. The animal poisons seem to kill primarily by circulatory collapse, a fact which might explain the peculiar resistance of the guinea pig to these venoms. An idea of the resistance of the guinea pig to the circulatory effects produced by anaphylaxis may be had if one prevents the occurrence of emphysema. This can be done by injecting a drug such as thymoxy-ethyl diethyl-amine (Rosenthal and Brown 14), which prevents the immobilization of the lung but is without effect on the circulatory symptoms produced by histamine. Sensitized guinea pigs previously injected with thymoxy-ethyl diethyl-amine always recover from a mild and transitory prostration, after the injection of the antigen. This shows that the circulatory effects of anaphylaxis in the guinea pig are very mild and apparently incapable of producing death. Considering the fact that rabbits, although resistant to anaphylaxis, frequently die by circulatory collapse produced by anaphylaxis, we may conclude that guinea pigs are even more resistant than rabbits to this form of circulatory impairment.

The foregoing facts may have some bearing on the general problem of toxemia and shock, since, in contradistinction to what happens in anaphylaxis, the toxic substances are more likely to enter the blood before reaching the tissues. As a matter of fact Dragstedt and Mead (15) were unable to demonstrate any increase of the histamine content of the blood after severe experimental surgical shock in the dog, and Rose and Browne (16) have shown a definite decrease of the histamine content of human blood after shock producing operations of several kinds. The foregoing experiments, performed with toxins which are known to liberate histamine, have shown that the experimental toxemia produced by direct intravenous injection of these poisons is likely to produce a decrease of the histamine content of the blood in the animal species studied. What part this decrease of histamine content might play in the production of the symptoms caused by these poisons is of course a matter of conjecture.

SUMMARY AND CONCLUSIONS

The venom of the rattlesnake (Crotalus atrox) and trypsin (crystalline preparation), when injected into guinea pigs, produced lethal shock and marked hyperemia of the abdominal viscera, but only moderate pulmonary emphysema. Adrenalectomy definitely increased the emphysema but apparently did not have any appreciable effect on the hyperemia of the abdominal viscera.

When injected into normal guinea pigs, crotalin and trypsin decreased the histamine content of the blood.

When injected into normal rabbits, crotalin (Crotalus adamanteus) in amounts sufficient to produce death in fifteen to twenty minutes caused a marked reduction of the histamine content of the blood. After injection of doses that caused almost immediate death, the histamine content of the blood was not reduced so

profoundly. The reduction of histamine was similar to that found after anaphylactic shock or an injection of trypsin.

The relation between these facts and what occurs in anaphylactic shock in guinea pigs and rabbits as well as the bearing of these facts on the general problem of toxemia and histamine content of the blood also has been discussed.

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THE EFFECT OF ADRENAL STEROIDS IN WATER INTOXICATION[1]

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The susceptibility of adrenalectomized and hypophysectomized animals to the intoxicating effects of excess water, and the replacement value of cortical extracts has been well demonstrated (1–5). Few extensive studies, however, on the use of crystalline adrenal steroids in protection against water intoxication have been made. Earlier work showed that estrogens, androgens and progesterone were ineffective in rats (4) and more recently that desoxycorticosterone acetate (referred to hereafter as DCA) will protect the adrenalectomized dog (6).

A study of the comparative potency of various adrenal preparations in protecting against excess water in adrenalectomized rats is reported here.

METHODS. The technique and type of animal used were the same as in previous work (5). Briefly, the tests consisted of giving male rats, adrenalectomized for 18 hours, and fasted for 12 hours, 6 per cent of their body weight of water at five successive hourly intervals. The rate of excretion of the administered water, and the occurrence of prostration, convulsions or death were the criteria of response. No animal was used for more than one test. In comparable studies made by others on the dog the tests were standardized differently, in that the minimal amount of water necessary to produce intoxication symptoms was determined (6).

DCA was used in sesame oil at a concentration of either 2 or 4 mgm. per cubic centimeter. Compound E was dissolved in acetone, sesame oil added and most of the acetone then removed to make a concentration of 4 mgm. per cubic centimeter. Adrenalectomized controls received these solvents in part of the cases without varying results.

The compound E (17-hydroxy-11-dehydrocorticosterone), amorphous fraction, and unfractionated extract of the adrenal cortex in aqueous solution were prepared in the laboratories of Dr. E. C. Kendall. Desoxycorticosterone acetate (Cortate) was kindly supplied by Dr. Erwin Schwenk, Schering Corporation and Eschatin by Dr. Oliver Kamm, Parke-Davis & Company. The extract of the adrenal cortex in oil was prepared for us at Princeton University.

Divided doses of the hormones in oil, given subcutaneously, were used unless otherwise stated. Ten per cent of the total dose was injected immediately

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after operation, 70 per cent one hour before water was given, and 20 per cent with the fourth dose of water. Slight variations in this procedure did not vary results. Large single injections of DCA were almost equally effective at 1 or 15 hours before administration of water, but less effective than the same treatment in divided doses (fig. 1, col. 6, 7, 3). Related experiments (not included in figure) indicated that with smaller doses of DCA a better effect was obtained with a single injection if given at 1 hour rather than 15 hours before water was given.

Hormones in aqueous solution were given intraperitoneally as follows: 10 per cent of the total dose immediately after operation, with the remainder

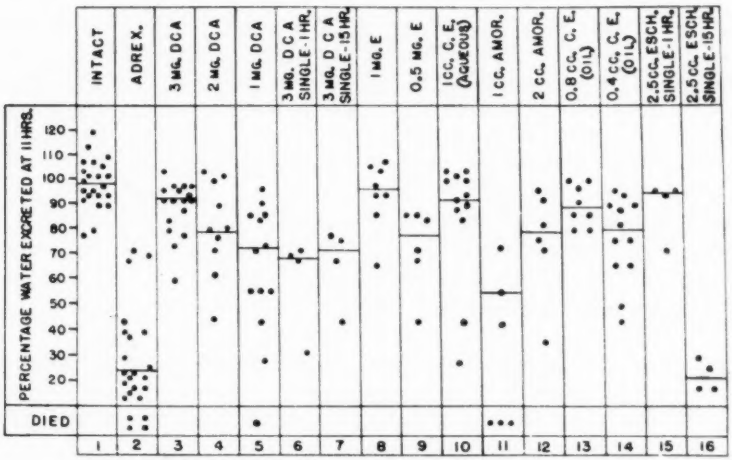

Fig. 1. Graph showing the percentage excretion of standard doses of water, given by stomach tube, to variously-treated rats. The numbers along the abscissa are for column identification. All animals were adrenalectomized except those in column 1. Each dot represents an individual animal. All treatments were given in divided doses except for those marked "single." The location of the dots for animals that died are not in scale to represent their water excretion. The solid line across each column locates the median figure for the group. DCA = desoxycorticosterone acetate; C.E. = unfractionated adrenal cortical extract; Esch = Eschatin.

divided into four equal portions given at the same time as the first four doses of water. Large single doses of Eschatin were effective if given 1 hour before the water, but not at 15 hours (fig. 1, col. 15, 16).

Characteristics of water intoxication in rats. The dose of water used in these tests was not sufficient to cause any symptoms of intoxication in normal rats, in which somewhat more than 90 per cent of the water given could generally be recovered as urine by 11 hours (fig. 1, col. 1). The output of urine was measured at regular intervals throughout a 24-hour period, but the total excretion of water at the 11th hour was as good an index of response as the whole 24-hour curve, and is the figure illustrated here.

Untreated adrenalectomized rats, without exception in our experience, were unable to excrete the water at a normal rate (fig. 1, col. 2) and in most cases either died or were prostrated or convulsive. A replacement therapy was considered complete if the animals were free from these symptoms and had excreted approximately 90 per cent or more of the administered water by 11 hours.

Quantitative studies were difficult in adrenalectomized animals, because of variable individual sensitivities to the administration of water. We have not been able to eliminate this by control of experimental conditions. In a group of animals that was almost completely protected by the hormones of the adrenal cortex there would generally be some which were little benefited. This accounts for the ranges noted in the graph. More rarely an abnormally high excretion of water was exhibited. In view of these facts the median was considered a more correct expression of average results than the mean. As would be expected, the variability increased as dosages of protective substances approached minimal effective levels. Despite the variability, however, dose-response relationships for any given preparation were always in consistent order, when expressed either in mean or median terms, even with the necessarily small number of animals used with the rarer substances.

Effects of unfractionated cortical extract in oil. This preparation was tested both for its influence in maintaining life and growth in thirty-day-old adrenalectomized male rats and for protection against excess water. The dose necessary to maintain a mean growth of 1.5 gram per day and life in 80 per cent of the individuals for ten days lay between 0.05 and 0.1 cc. daily, probably nearer the former. Eight-tenths cubic centimeter was the minimal amount required for complete protection against water intoxication (fig. 1, col. 13, 14). In other words, the dose required to protect against water intoxication approximated 10 times the daily life-maintaining dose.

Effects of desoxycorticosterone acetate. DCA was likewise compared for its ability to protect against excess water and for its effect on life-maintenance. We found that the minimal dose for life maintenance approximated 0.05 mgm. DCA daily. If its effectiveness in water intoxication had been in the same ratio to life and growth maintenance as that of the extract of the adrenal cortex (10:1) then 0.5 mgm. DCA should have prevented symptoms of water intoxication. This was found not to be true. It was only with a dose of 3 mgm. DCA that we could get a resistance to excess water which approached normal, although lower doses were distinctly beneficial (fig. 1, col. 3, 4, 5).

To state the above results in simpler equivalents: 1 mgm. of DCA was somewhat better than 1 cc. of cortical extract for maintenance of life, but in protection against water about 4 mgm. DCA were needed to exert the effect of 1 cc. of the extract.

We considered the possibility that the relative ineffectiveness of DCA in water intoxication was due to the fact that it was slowly absorbed.

All animals were kept for a week after the experiment, if they lived that long, to see if those treated with DCA lived and remained free of insufficiency longer

than those treated with substances more effective in the acute water test. No differences were noted.

Effect of compound E (17-hydroxy-11-dehydrocorticosterone). This compound, tested at two dose levels, was highly efficient in protection against excess water (fig. 1, col. 8, 9). One milligram gave the best replacement we have obtained with any substance, and was probably better than 3 mgm. of DCA.

Effect of amorphous fraction and cortical extract in aqueous solution. These two preparations were of approximately equal potency for the maintenance of adrenalectomized dogs—1 cc. daily being needed. It has been established that in several other specific effects the amorphous fraction is weak or lacking in activity (7). The whole extract gave full replacement against water intoxication with a 1 cc. dose (1 cc. = 75 grams fresh glands) (fig. 1, col. 10). At a similar dose level, the amorphous fraction was ineffective, as indicated by death of half of the animals and a poor water excretion in the survivors. At a 2 cc. dosage the amorphous fraction exerted a beneficial effect, but one decidedly inferior to either 1 cc. of the extract or 1 mgm. compound E (fig. 1, col. 11, 12).

Discussion. These water-intoxication tests demonstrate quantitatively different effects of various adrenal cortical substances. These differences were, however, only ones of degree, as all preparations were helpful at some dose level. It would seem that protection against the toxic effects of water is more effectively provided by those compounds with an atom of oxygen at C_{11}. These compounds are also highly active in stimulating gluconeogenesis, in the maintenance of muscular efficiency, in causing thymic atrophy, and in the support of lactation, but are relatively weak in the maintenance of life, growth, electrolyte balance and renal function (7). It might be expected that the compounds more active in the latter category, such as desoxycorticosterone and the amorphous fraction, would be the ones most effective in protecting against excess water; and the basis for a correlation of the water-intoxication responses with the special sphere of activity of the oxygen-at-C_{11} compounds is not clear. This is probably because the nature of the basic derangement in water intoxication is not well established.

SUMMARY

The effects of various cortical extracts and compounds in protecting adrenalectomized animals against water intoxication were studied, and the following conclusions drawn:

1. When compared to a whole extract of the adrenal cortex, desoxycorticosterone is relatively less effective in protection against excess water than in the maintenance of life and growth.

2. Compound E was at least three times as efficient as desoxycorticosterone acetate in protecting against excess water.

3. The amorphous fraction was only weakly effective in preventing water intoxication when compared with a whole extract of equal potency in maintaining life in the adrenalectomized dog.

4. All substances used were, however, beneficial if adequate doses were given.

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THE EXCRETION OF RESPIRATORY TRACT FLUID

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Direct proof is lacking for the commonly made statement that fluid material produced in the respiratory airway (respiratory tract fluid or R.T.F.) is normally carried up and excreted through the trachea. That such a mechanism does exist has been deduced from a number of observations. The tools exist for just such a job, as ciliated mucosa lining the airway, the presence of the bronchial muscles of Reisseisen, cough and churning respiratory movements. A number of foreign materials have been shown to be excreted from the lungs via the trachea, such as dyes, silica and certain parasites. The cilia-propelled upward passage of particles such as bits of cork has been visualized. These are all well known facts and upon them and related evidence, it has been concluded that normal R.T.F. is excreted up the trachea.

Nevertheless this has not been proven, apart from coughed up sputum which may not be regarded as normal or universal, because R.T.F. has never been quantitatively collected from the trachea and measured under conditions which would prove that there is an upward excretion. A method for collecting R.T.F. has been recently described by Boyd and Perry (4). In this method, animals are anesthetized with ethyl carbamate, a tracheal cannula is inserted and the animal partially suspended upside down to allow R.T.F. to *drain* out of the respiratory system. Under these conditions, an appreciable amount of R.T.F. could be collected from cats and rabbits but the experiment did not prove that R.T.F. was being carried up to the trachea in these animals. To investigate this probability, we have compared the output of R.T.F. drained from the lungs of cats which were suspended upside down with the output in cats lying in the prone position, the normal position of the axis of the body in this species. A diagram of the arrangement is shown in figure 1.

The animals were anesthetized with ethyl carbamate intraperitoneally supplemented with procaine hydrochloride locally. A side-armed cannula was inserted into the trachea and connected with a collecting tube and an air conditioning unit as illustrated diagrammatically in figure 2. The air conditioning unit warmed the inhaled air to body temperature and saturated it with water vapour. To accomplish this, air passed over trays of cotton kept continuously moist with dripping water and warmed by electric bulbs. The efficacy of the apparatus was tested from time to time by drawing the air from the conditioner into a chamber and measuring its temperature and relative humidity. All exposed parts were insulated to prevent condensation of water vapour. A num-

ber of experiments were performed to ascertain that the fluid collected and called R.T.F. was not condensed water vapour; of these, the most striking evidence in proof was the occasional collecting of no R.T.F. over a period of some hours, which would be impossible if water vapour were condensing and collecting in the receiving tube.

The experiments described herein were performed in the summer months. During this season, the output of R.T.F. is greater than in the winter months (4) and the lungs and trachea contain more water than they do in the winter (1, 2).

The animals were attached vertically head downward, or nearly vertically, for several hours and the output of R.T.F. per kilo body weight per hour determined. Then they were placed prone upon their bellies for several hours and the output of R.T.F. again measured. In other instances, the order of procedure was reversed, beginning with the animals prone and then inverted, so that approxi-

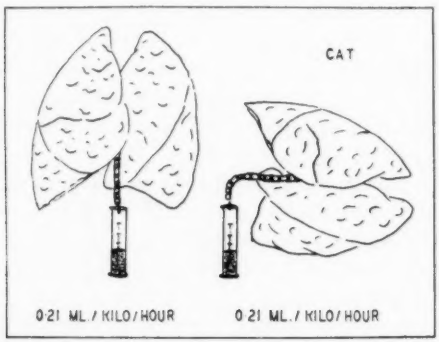

Fig. 1

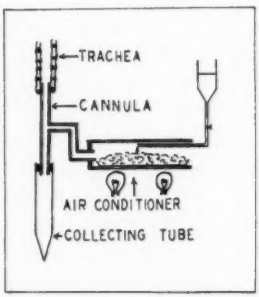

Fig. 2

Fig. 1. A diagrammatic representation of the excretion of respiratory tract fluid by the lung of the cat in the inverted versus the prone position.

Fig. 2. A diagrammatic representation of the method of collecting respiratory tract fluid.

mately half the animals were started in the prone and half in the inverted position.

The average results in 15 such experiments in cats have been summarized in table 1. In 5 instances cats in the prone position put out more R.T.F. than in the inverted position. In 3 instances the output was the same in both positions and in 7 instances cats excreted less in the prone than in the inverted position. It is obvious from these data that the position of the cats was not a factor influencing the output of R.T.F. Thus, when the data were averaged, it happened that the mean output per kilo per hour turned out to be exactly the same in each case. The mean value was 0.21 ml. per kilo body weight per hour as shown in table 1 and figure 1.

Indirect evidence was obtained in the rabbit confirming these results in the cat. For other reasons, no direct comparison was made with rabbits in the prone and then in the inverted position on the same day. In May, 1941, ex-

periments were performed on a total of 24 rabbits arranged in the inverted position and the mean output of R.T.F. was 0.10 ml. per kilo per hour. In July, 1941, similar experiments were performed on 10 rabbits arranged in the prone position and the mean output of R.T.F. was 0.11 ml. per kilo per hour and in early September, 1941, a further 12 experiments were done on rabbits in the prone position with a mean output of 0.12 ml. per kilo per hour. Thus the average of 24 rabbits arranged in the inverted position was 0.10 ml. per kilo per hour and of 22 rabbits arranged in the prone position was 0.12 ml. per kilo per hour. These two means are not strictly comparable on a postural basis since an interval of several weeks ensued between them and there may have been some variation due to season. Any seasonal variation at this time of the year would probably be of a minor degree because Boyd and Johnston (1, 2) found no marked changes in lung and tracheal water from late spring until the autumn. In view of these considerations, it seems reasonable to

TABLE 1

A comparison of the hourly output of respiratory tract fluid (ml. per kilo body weight per hour) of cats arranged in the prone and in the inverted positions

EXPERIMENT NUMBER	PRONE POSITION	INVERTED POSITION	EXPERIMENT NUMBER	PRONE POSITION	INVERTED POSITION
1	0.47	0.40	9	0.46	0.11
2	0.33	0.19	10	0.15	0.10
3	0.19	0.19	11	0.13	0.15
4	0.09	0.28	12	0.20	0.19
5	0.05	0.05	13	0.16	0.19
6	0.05	0.07	14	0.38	0.38
7	0.15	0.20	15	0.21	0.44
8	0.08	0.20	Mean	0.21	0.21

conclude that in rabbits, as in cats, the output of R.T.F. is just as extensive with the animal arranged in the prone as in the inverted position.

What mechanisms are responsible for this excretion of R.T.F.? In a consideration of this question, the respiratory tract may be divided into two regions which may be termed *a*, the alveolized region, and *b*, the bronchialized region. The alveolized region consists of the pulmonary alveoli, the alveolar sacs, the atria, the alveolar ducts and the respiratory bronchioles, a region which may be considered embryologically as derived from mesoderm (8), which is histologically distinct and physiologically relegated primarily to the function of gaseous exchange. The bronchialized region extends from the terminal bronchioles of 0.5 mm. diameter up to and including the trachea; it is primarily of endodermal origin, anatomically distinct from the first region and physiologically acts as an air conducting and fluid secreting structure.

In the alveolar region there are no cilia and but occasional smooth muscle fibres (7). The velocity of air movement during coughing is probably insufficient to affect any fluids present; the velocity has not been measured in the lung alveoli but it has been estimated in the respiratory bronchiole to be of

the order of 3 miles an hour during coughing (6). Some of the fluids in the alveolized region are absorbed into the pulmonary circulation or into the lymphatics, either directly or via the intra-alveolar histiocytes and this may be the most effective means of removing such fluids (9). Gordonoff (5) believes that the churning movements of respiration are the most important mechanism for draining this part of the tract and, for that matter, of the respiratory airway in general. Policard (8) suggests another mechanism; he says that when air is drawn into and expands an alveolus to capacity, it must squeeze out any fluid present and force it up into the bronchiolar passages.

In the bronchialized region there are a number of mechanisms present which may function to excrete R.T.F. The lining of ciliated columnar epithelium is capable of propelling particles along at the rate of 2 to 5 cm. per minute (3). There is present a geodesic network of the bronchial muscle of Reisseisen which passes through a systole and diastole during expiration and inspiration and probably aids in the excretion of R.T.F. but whether in a peristalsis-like manner is open to question (6). The velocity of air expired during coughing becomes progressively greater as the bronchiolar diameter increases and it reaches a maximum of the order of 100 to 200 miles an hour in passing through the glottis (6). The R.T.F. is enriched with secretions from the calciform or goblet cells and the acinar bronchial glands. Finally the R.T.F. is at least kept mixed and churned by the mechanical movements of respiration.

These various mechanisms suffice to maintain a continuous upward streaming of R.T.F. In the cat and rabbit anesthetized with ethyl carbamate, they suffice to excrete between 1 and 20 ml. of R.T.F. per day. This would correspond on a body weight basis to the excretion of from 30 to 600 ml. per day by an average man.

SUMMARY

It was found that cats anesthetized with ethyl carbamate and tracheotomized excreted as much respiratory tract fluid (R.T.F.) in the prone as in the inverted position. The average excretion, measured in the summer months, was 0.21 ml. per kilo body weight per hour. This finding indicated the presence of an effective mechanism for the excretion of R.T.F. Confirmatory data were obtained in rabbits. The mechanism of excretion was discussed.

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MECHANISMS FOR THE MAINTENANCE OF LIFE IN THE NEWBORN DURING ANOXIA[1]

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In a previous publication the extraordinary tolerance of the newborn of various species to anoxia was described (1). Mature animals succumb after breathing undiluted nitrogen for approximately 3 minutes while the average values for newborn animals are: rats, 50 minutes; cats, 25 minutes; dogs, 23 minutes; rabbits, 17 minutes; and guinea pigs, 7 minutes. Various factors permitting this prolonged survival were discussed. These include *a*, degree of development at the time of birth, i.e., the guinea pig is more advanced than the rat; *b*, rate of cerebral metabolism which is lower in the infant than in the adult, and *c*, anaerobic sources of energy. The last of these factors is the subject of the present investigation. It is well established that the process of glycolysis may afford energy. The anaerobic breakdown of carbohydrate with a release of energy occurs in the series of transformations; glucose phosphate → triose phosphate → phosphoglyceric acid → pyruvic acid → lactic acid. These changes are inhibited by fluoride at phosphoglyceric acid and by iodoacetate at triose phosphate.

METHOD. As in the previous study, animals were placed in a jar in which nitrogen was substituted for air. Sodium iodoacetate, 0.18 mgm. per gram or sodium fluoride, 0.5 mgm. per gram, was injected subcutaneously. Both the carbohydrate stores and the level of the blood sugar were altered in different experiments, hypoglycemia being induced by the injection of insulin and hyperglycemia by the intraperitoneal injection of glucose. The lactic acid contents of the brain and of the whole animal were determined before and after exposure to nitrogen. Some of the animals were injected with iodoacetate and others served as controls. To stop glycolysis the isolated head or whole body was rapidly frozen in dry ice and ether. The frozen material was then triturated in a solution of 2 per cent iodoacetate and the lactic acid contents of the tissues and the suspending fluid were estimated by the method of Friedemann, Cotonio and Shaffer (2).

In order to dissociate the effects on brain from those on other organs, newborn

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[2] Deceased November 23, 1940.

rats were decapitated (3) and the duration of the gasping reflex of the isolated head determined. Iodoacetate or fluoride was injected in other newborns before decapitation and the effects of these drugs on the gasping reflex were studied. The influence of these drugs on the duration of life was observed in animals injected with the same amount of iodoacetate or fluoride, but not decapitated. Comparative studies were made in an atmosphere of 5 per cent CO_2 in N_2 and the CO_2 released by the newly formed lactic acid was measured in the Warburg apparatus. Cerebral tissues excised from newborn and adult rats were used for these studies.

Results. The concentrations of iodoacetate and fluoride used permitted survival of the newborn rats for approximately 50 minutes when breathing air (table 1). Other rats injected with these same inhibitors and respiring nitrogen lived on an average 3 minutes with iodoacetate, and 16 minutes with fluoride before they succumbed in rigor. The effects of the same dosage of these drugs on the duration of the gasping reflex of the isolated head are included in table 1. It may be seen that the gasping period is shortened by previous injection of either iodoacetate or fluoride.

Table 2 presents the increase in lactic acid contents of the animals that survived in nitrogen for 50 minutes. Those animals injected with iodoacetate succumbed with no increase of lactic acid. Each value represents the average of 9 observations.

In table 3 are disclosed the increases of lactic acid in the brain of newborn rats subjected to nitrogen.

The observations on glycolysis of the excised cerebral tissues yielded an average value of 215 cu. mm. of carbon dioxide or 0.86 mgm. of lactic acid per 100 mgm. of tissue in 27 experiments on adults. The average of 26 experiments on newborns was 100 cu. mm. of carbon dioxide or 0.4 mgm. of lactic acid per 100 mgm. of tissue per hour.

Table 4 reveals the effects of hyperglycemia and hypoglycemia on the survival time in nitrogen.

Discussion. The results of these experiments stress the importance of carbohydrate as an anaerobic source of energy for the young. The general increase of lactic acid in the body is the resultant of the anaerobic cleavage of carbohydrate in the various parts; striated and cardiac muscle, brain and other organs with the probable exception of the liver. The rise of lactic acid (table 3) in the brain is due only in part to diffusion from the blood. A portion of the cerebral lactic acid is produced by the anaerobic splitting of carbohydrate in that organ. As shown by Holmes and Holmes (4) the concentration of cerebral lactic acid developed under anaerobic conditions is proportional with the level of blood sugar and the latter is increased as a result of glycogenolysis in the liver. A portion of the lactic acid may be a result of the breakdown of brain glycogen.

If carbohydrate is important as an anaerobic source of energy, alterations in the amount of this substance should change the survival period. In those experiments in which the carbohydrate stores of the body were raised by the intraperitoneal injection of glucose, the increased duration of survival may be at-

TABLE 1

Effect of iodoacetate and sodium fluoride on survival time and gasping reflex of newborn rats

	MGM. PER GM.	AVERAGE SURVIVAL TIME OF INTACT ANIMAL IN AIR	AVERAGE SURVIVAL TIME OF INTACT ANIMAL IN NITROGEN	AVERAGE GASPING TIME OF ISOLATED HEAD
		minutes	*minutes*	*minutes*
Iodoacetate	0.18	50	3	2½
Sodium fluoride	0.5	50	16	7
Uninjected control		Continues to live	50	20

TABLE 2

Iodoacetate and anoxia in newborn rats

CONDITIONS	AVERAGE SURVIVAL TIME	AVERAGE LACTIC ACID
	minutes	*mgm. %*
Iodoacetate in air	50	41
Iodoacetate in nitrogen	3	38
Nitrogen only	50	145*

* Determined after 40 minutes in nitrogen.

TABLE 3

Lactic acid increase in brain of newborn rats subjected to nitrogen

AGE	LACTIC ACID BEFORE ANOXIA	LACTIC ACID AFTER ANOXIA	PERIOD OF ANOXIA
days	*mgm. %*	*mgm. %*	*minutes*
1	63	182	40
1	55	180	40
1	50	186	40
7	29	117	20
8	26	116	20

TABLE 4

CONDITIONS	SURVIVAL TIME	AGE OF RATS
Hypoglycemia and anoxia		
	minutes	
Anoxia	50	Newborn
Anoxia + insulin hypoglycemia	20	Newborn
Hyperglycemia and anoxia		
Anoxia	16	8 days
Anoxia + glucose	30	8 days

tributed to the augmentation of this anaerobic source of energy. This could be demonstrated better in rats 8 days old than in the newborn for the former survive

for the shorter period of 16 minutes of anoxia, and excessive hyperglycemia, too prolonged, results in convulsions and death. The longer survival of newborn rats affords the best opportunity to study a decrease in this period. When animals were subjected to hypoglycemia and anoxia, the duration of the anaerobic survival was shortened (5). Many other workers have studied the simultaneous effects of hypoxia and alterations of blood sugar level in mature animals and men. A synergistic action of hypoglycemia and anoxia has been emphasized by Gellhorn (6). McFarland and Forbes (7) also reported that the administration of sugar may overcome, to some extent, the visual disabilities produced by anoxia, while hypoglycemia may accentuate them.

The experiments with iodoacetate and fluoride demonstrate the importance of carbohydrate as an anaerobic source of energy (8). Concentrations of these drugs adequate to inhibit glycolysis shorten the survival period when the infants are placed in an atmosphere of nitrogen, but nevertheless permit life in air for a considerable time.

The experiments on the duration of the gasping reflex reveal that both iodoacetate and fluoride exert an inhibitory effect on the anaerobic processes which produce the energy necessary for gasping. The results obtained with the isolated head are not influenced secondarily by changes of other parts of the body. For example, after administration either of iodoacetate or fluoride and exposure to nitrogen the heart stops practically simultaneously with respiration. Without these inhibitors the heart continues beating for some time after respiration has ceased.

From a great volume of previous work it has been disclosed that the brain is a limiting factor in tolerance to anoxia. The infant possesses a rate of cerebral metabolism and oxygen utilization lower than that of the adult (9, 10, 11) thus requiring a smaller amount of energy for the maintenance of function and life. Observations in which the metabolic rate was raised by increasing the environmental temperature of the poikilothermic newborn rats from 24° to 34° disclosed a shorter anoxic survival period. This increase in temperature must necessarily raise the requirements of the brain. The rate of glycolysis (12) is increased at the higher temperatures. This augmented glycolysis causes a a more rapid utilization of the available carbohydrate.

Experiments, in which glycolysis was studied in adult and infant excised cerebral tissues at a temperature of 38°C, demonstrated a much lower rate in the infant. This low metabolism, however, is adequate to maintain the lesser requirements of the infant brain as evidenced by the continued function of the respiratory centers in the absence of oxygen. It should be mentioned, however, that in the adult neither lowering the metabolic rate by freezing nor raising the carbohydrate stores by the injection of glucose alters significantly the short survival period in nitrogen.

SUMMARY

The importance of carbohydrate as an anaerobic store of energy has been studied in the newborn rat. An increase of the carbohydrate stores permits

longer survival in nitrogen while a decrease has the reverse effect. The injection of iodoacetate or fluoride shortens the survival period in nitrogen, presumably by preventing the breakdown of carbohydrate. The rate of cerebral glycolysis in the newborn is slower than that of the adult. This slow rate appears adequate to support the lower metabolic demands of the infant brain for a considerable period in the absence of oxygen.

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DIETARY CONTROL OF THE WATER CONTENT OF THE SKIN OF THE ALBINO RAT[1]

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Therapeutic practice based on the assumption that the water content of the tissues is increased by a diet rich in carbohydrate and reduced by one containing a large amount of fat (1–6), add a practical to the theoretical interest attached to the problem of the dietary control of tissue hydration. It has been shown experimentally (7) that neither a high carbohydrate nor a high fat ration produce changes of any appreciable magnitude in the amount of water contained in the various organs of the albino rat. The water content of the skin, however, was not determined. Since it is possible that changes in the diet might affect the hydration of the skin, as would appear to be the case from the observations of Adlersburg and Porges on man (1), the present study was undertaken to supplement our previous experiments. Although the primary interest was the effect of a high carbohydrate-low fat diet as compared with one containing a relatively small amount of carbohydrate and a large proportion of fat, the effect of a well diversified stock diet was also studied. Analyses were made of the fat, nitrogen and glycogen content of the skin to ascertain whether there was any correlation of these constituents on the one hand with the type of diet and on the other with the water content of the skin.

METHOD. Eight groups of triplicate litter mates of male albino rats and the same number of females were selected at weaning and fed a food mixture similar to the Wistar-Dry Ration for ten days. At the end of this period they were placed on the experimental rations. One animal in each group was fed a stock ration (7) supplemented by Purina dog chow, lettuce, cabbage, milk and hard boiled eggs; the second a high carbohydrate, and the third a high fat ration. The carbohydrate ration contained 70 per cent sucrose and the fat ration 50 per cent lard and 20 per cent sucrose, the remainder of both rations consisting of 15 per cent casein, 10 per cent Fleischmann's yeast and 5 per cent Osborne-Mendel salt mixture. Each animal in the three groups was given daily seven drops of a 6:1 cod liver oil-linoleic acid mixture by medicine dropper. Sufficient food and water were at their disposal at all times to allow them to eat and drink *ad libitum*.

The animals were kept on the different rations for 70 days and at the conclusion of this period fasted 24 hours with free access to water and then de-

[1] Preliminary report: This Journal **133**: P. 311, 1941.

capitated. Approximately 5 cc. blood was collected in a weighing bottle from the bleeding animals for determination of its water content. A portion of skin was quickly taken from the back and after removal of adherent fat, was divided into several parts for analysis of the water, glycogen, fat, and nitrogen content. The body was then chopped with a cleaver, ground in a sausage grinder and finally passed through a corn mill. This procedure, which was carried out in a closed room saturated with water vapor, was found by analysis of aliquots to yield a homogeneous mass. Duplicate determinations of the water content were made on portions of 5 to 8 grams of the ground material, which was dried

TABLE 1

*Water content and other constituents of the skin of albino rats on various rations**

RATION	MALES				FEMALES			
	Water	Fat**	Protein (N × 6.25)	Glycogen	Water	Fat**	Protein (N × 6.25)	Glycogen
	Per cent of wet weight				Per cent of wet weight			
Stock	60.3	6.6	29.6	0.073	51.1	12.0	25.0	0.064
High carbohydrate	55.1	9.3	28.3	0.069	43.7	20.1	23.5	0.045
High fat	51.4	17.6	24.5	0.075	39.2	31.6	19.4	0.050

* Each value is an average of 8 experiments.

** As fatty acid.

TABLE 2

*Body weight and water content of the body and of the blood of albino rats on various rations**

RATION	MALES				FEMALES			
	Initial weight	Final weight	Water content of body	Water content of blood	Initial weight	Final weight	Water content of body	Water content of blood
			Per cent of wet weight				Per cent of wet weight	
Stock	36	211	63.1	78.5	41	173	61.5	78.4
High carbohydrate	37	250	58.7	80.1	40	190	56.8	80.1
High fat	37	273	50.9	80.4	40	214	48.6	80.3

* Each value is an average of 8 experiments.

in a desiccator for 48 hours over $CaCl_2$ and finally to constant weight over P_2O_5. The water content of the skin and blood was obtained by the same procedure. The fat content was determined (as fatty acid) by the method of Leathes and Raper (8) as described by Feyder (9); nitrogen by the Macro-Kjeldahl method, and glycogen by the Good, Kramer and Somogyi procedure (10).

RESULTS. The analytical data of the experiments are presented in tables 1 and 2. Ketosis played no part in the experimental results for it has been shown (7) that ketosis is not produced in the rat by the fat ration fed in the present experiments.

From inspection of table 1 a sex difference in the composition of the skin is

readily discernible. On all three rations the skin of the females contained a definitely lower percentage of water and of protein (N × 6.25) and a higher percentage of fat than the skin of the males. While the difference in the percentage protein between the two sexes was smaller than that of water and of fat, it was nevertheless statistically significant. The probability of the difference being a chance occurrence as calculated by Fisher's method (11) was 0.004 on the stock ration and less than 0.0001 on the other two rations. In no instance was the percentage protein in the skin of the males as low as the average for the females and, conversely, no single value for the females as high as the average for the males. The average glycogen content of the skin was lower in the females than in the males on each of the three rations but the difference between the two sexes was not statistically significant except on the carbohydrate and fat rations. The actual amount of glycogen in the skin was so small that the maximum average difference between the two sexes is probably of no practical consequence.

The percentage water content of the skin of both the males and females was lower on the fat than on the carbohydrate ration and in turn lower on the carbohydrate than on the stock ration. The differences on the three rations are large enough to be of practical importance and are all statistically significant. In each of the sixteen groups the percentage water on the fat and carbohydrate rations was lower than on the stock ration. The individual differences between the percentages on the fat and carbohydrate rations were also consistent, for in 15 of the 16 groups they were lower on the fat ration.

The amount of fat deposited in the skin was definitely increased by feeding a large amount of fat. On the carbohydrate ration the fat content of the skin was intermediate between that which obtained on the other two rations. As in the case of the water content the differences in the percentage fat on the three rations shown in table 1 are of appreciable magnitude and are statistically significant. In every instance the percentage was higher in the fat fed animals than in the litter mates on the carbohydrate and stock rations; and in 14 of the 16 groups it was higher on the carbohydrate than on the stock ration. The increase in the fat content of the skin was accompanied by an increase in body weight (table 2).

An inverse relationship between the water and fat content of the skin is obvious from the data presented in table 1. The correlation coefficient for the males was -0.77 and for the females -0.70 with probable errors of ± 0.08 and ± 0.07, respectively.

With an increase in the average fat content of the skin on the various rations there was a decline in the protein content. In both sexes the percentage protein of the skin on the stock ration was approximately 1.5 and 5.0 per cent higher than on the carbohydrate and fat rations, respectively. Both differences were statistically significant. The value of P for the smaller difference was 0.006. In each of the 16 groups the percentage protein was lower on the fat than on the stock ration, and in 13 out of 16 groups it was lower on the carbohydrate as compared with the stock ration.

The decrease in the percentage protein on the various rations which occurred with the increase in the percentage fat would lead one to surmise a positive correlation between the water and protein content of the skin in contrast to the negative correlation between the water and fat content. Calculations revealed a fairly high correlation. The coefficient was +0.57 (P.E. ±0.09) for the females and +0.61 (P.E. ±0.09) for the males.

The negative correlation of the percentage water content of the skin with the fat content and the positive correlation with the protein content raises the question: Are the differences in the water content of the skin on the various rations to be attributed to the differences in the fat or to differences in the protein content? The following solution of the problem is offered: On the assumption that the fat in the skin contained an inappreciable amount of water, the percentage fat in each experiment was subtracted from 100 to obtain the amount of fat-free material in the tissue. This assumption we believe to be justified inasmuch as Fenn and Haege (12) in their studies on the liver found that lipid is deposited in this organ without water. Kaplan and Chaikoff (13) had previously reported that only a minute amount of water accompanies the deposition of lipid. As the amount of water in each gram of skin was known, the next step was to calculate what the percentage of water would have been if there were no fat present. The values thus obtained were 64.7, 60.8 and 62.3 per cent water for the skin of the males on the stock, carbohydrate and fat rations, respectively, and 58.1, 54.6 and 57.5 for that of the females. Percentages of the protein content similarly calculated on a fat-free basis were found to be 31.7, 31.2 and 29.7 for the males and 28.4, 29.4 and 28.4 for the females. Correlation coefficients derived from these water and protein percentages of fat-free tissue proved to be extremely low and of no significance. The coefficient for the data on the males was 0.03 and for those on the females 0.39, with a probable error of 0.12 for the latter. It would appear, therefore, that the correlations between the water and protein content of the skin obtained from the original data, the averages of which are given in table 1, were "reflected" and not "true" correlations. These considerations lead to the conclusion that differences in the percentage water of the skin on the various rations should be attributed to differences in the fat and not to differences in the percentage protein of the tissue. It becomes apparent that the diet may affect the percentage water content of the skin by increasing or decreasing the fat deposition in the tissue.

The mechanism whereby the amount of water in the skin is made to vary inversely with the amount of fat deposited may be either chemical or physical in character. The former would obtain if the fat should become an integral part of the tissue thereby changing the chemical composition and hydration capacity of the tissue cells; the latter, if the fat were to change the percentage of water by mere superposition on the cellular substance. The manner in which the latter mechanism might operate may be shown by a simple illustration. If 0.2 gram fat which contains no water were added to a gram of tissue containing 0.7 gram water, 0.04 gram protein, and 0.26 gram fat and other substances, the percentage water would be reduced from 70 to approximately 58, although the

chemical composition and water content of the non-fat portion of the tissue had not changed. In our opinion this mechanism probably accounts for the inverse relationship of the percentage fat and percentage water content of the skin. This opinion is based on the small differences in percentage water calculated on the fat-free basis in the manner described in the preceding paragraph, as compared with the much larger differences shown in table 1. It is recognized, however, that the evidence at hand is not conclusive and it is possible that both the chemical and physical mechanisms may be operative. A final decision must await further investigation.

Since the addition of fat to the tissue can change the percentage protein and water by the weight factor alone without affecting the chemical composition of the cellular substance, it is possible that the differences in the fat content of the skin of the males and females may have produced in this manner the differences in the percentage protein and water of the skin of the two sexes. In this event the differences in protein and water would not have been true sex differences but would have merely reflected the sex difference in the fat content of the skin. That such was not the case becomes evident upon inspection of the values for the percentage protein and water calculated on a fat free basis. It will be observed that in these derived values a sex difference persists on all three rations. Mathematical analysis showed that the differences were all statistically significant. The difference in the percentage protein and water as well as the percentage fat of the skin therefore represents a true sex difference.

The various rations had an effect on the percentage water of the entire body corresponding to that on the skin (*cf.* tables 1 and 2). This effect is doubtless due to the differences in the percentage water of the skin on the three rations, for it has been shown (7) that differences in the water content of the muscle and various internal organs on the rations used in these experiments are too small to have an appreciable effect on the percentage water in the entire body.

An interesting observation of these experiments for which at present we can offer no satisfactory explanation is the change in water content of the blood induced by the carbohydrate and fat rations. On both these rations the blood contained the same amount of water which in turn was significantly higher than on the stock ration. The values on the carbohydrate and fat rations were higher than those on the stock ration in each of the 16 groups.

CONCLUSIONS

A sex difference obtains in the percentage fat, protein and water of the skin of albino rats. In the females the fat content of the skin is higher while the percentage protein and water is lower than in the males.

The percentage water content of the skin of both sexes was found to be appreciably lower on a high fat than on a high carbohydrate ration, and lower on a carbohydrate than on a well diversified stock ration.

Differences in the percentage water content of the skin on the various rations were reflected in the total percentage water content of the body.

The percentage water in the skin was in inverse relationship to the percentage fat and in direct relationship to the percentage protein.

It is concluded that the positive correlation between the percentage water and percentage protein was not a true correlation but was the resultant of variations in the fat content. The differences in the percentage water of the skin on the various rations are attributed to differences in the deposition of fat in the tissue.

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EFFECT OF PURIFIED PITUITARY PREPARATIONS ON LIVER WEIGHTS OF HYPOPHYSECTOMIZED RATS[1]

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The great variety of changes which are observed after hypophysectomy or can be produced by administration of pituitary extracts has led to an equally great number of postulated "hormones." The existence of only six of these, however, can be regarded as established by their isolation and purification in several laboratories. Methods which lead to highly purified preparations of these six hormones have been worked out in this laboratory; this has made possible a systematic investigation to determine how far certain metabolic effects of crude pituitary extracts can be attributed to any of the accepted six or must be due to separate factors yet to be purified. Of the hormones at our disposal two appear to be chemically pure, lactogenic hormone (1) and one of the gonadotrophins, interstitial cell stimulating hormone (ICSH) (2). The other gonadotrophin, follicle stimulating hormone (FSH) (3) at its present state of purification reveals its specific effect at doses of 0.003 mgm. and appears to be contaminated with ICSH but with none of the other four hormones.[2] Thyrotrophic hormone (4) exerts its specific effect at about 0.01 mgm. and appears to be contaminated with ICSH and a very small amount of growth hormone.[3] Growth hormone, active also at about 0.01 mgm. contains adrenocorticotrophic hormone but no other established hormone (5).[4] Adrenocorticotrophic hormone is the least purified, at least in the chemical sense. While comparatively great doses of this hormone are needed for adrenal stimulation (0.05 mgm. daily in hypophy-

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[2] Contaminations with traces (i.e., less than 1 per cent) would escape most present methods of bioassay; while it is recognized that such traces of contaminating hormones may be present in all hormone preparations isolated from the pituitary, it appears unlikely that they exert any biological activity at such low levels and they will be disregarded in this discussion.

[3] In view of the fact that thyroxin at certain levels causes a limited weight gain in young hypophysectomized rats, it is difficult to say how far the slight weight gain produced by high doses of thyrotrophic hormone may be due to growth hormone contamination, and how far to stimulation of the thyroid.

[4] Recent advances in the purification of adrenocorticotrophic and growth hormones achieved by Doctors Li and Marx have considerably reduced their respective contaminants; such preparations will be used as far as possible in future work.

sectomized rats), it proves to be appreciably contaminated only with lactogenic hormone.[4] The approximate state of biological purity of the various hormone fractions employed during this and the ensuing investigations will be illustrated by figure 1.

It has long been known that "after hypophysectomy the percentage loss in weight by the liver is somewhat greater than that of the whole body and that in growth hormone-treated rats, the liver gains more in weight, relatively, than the rest of the body" (6). The nature of the increase in liver weight following administration of growth hormone to normal rats has been studied by Lee in some detail (6, 7). It was found to represent a true growth effect, i.e., the livers contained absolutely more cells, more nitrogen, more water and less fat. By use of the paired feeding technique it was also established that this effect was not due to an increased food intake of the treated rats. The growth hormone preparations used were not characterized in regard to their freedom from or

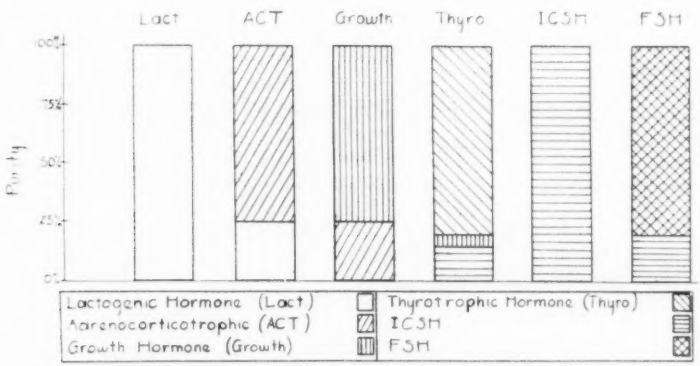

Fig. 1. State of purity of anterior pituitary hormones employed for metabolic studies. Approximate degree and nature of contamination.[5]

contamination with other active principles of the pituitary. This appears of importance in view of the growth stimulating effect of a single high dose of thyroxin on the livers of rats, noted by Sternheimer (8). This brought up the question as to whether the liver growth effects produced by Lee with pituitary extracts might not be mediated by the thyroid gland.

METHODS. Groups of 8 to 10 hypophysectomized female rats, 26 to 28 days at operation and 6 to 8 days (in one experiment 18 days) postoperative were injected daily (except Sundays) for 10 days with 0.5 cc of the hormone solution. Thyroxin was injected subcutaneously, all pituitary preparations intraperitoneally.[6] The rats were fasted for 5 to 6 hours before autopsy.

RESULTS. *I. Pituitary preparations.* Of the pituitary hormone preparations

[5] Further purification is in progress and has been particularly successful in regard to adrenocorticotrophic and growth hormones.

[6] Similar results were also obtained upon subcutaneous administration.

studied, only growth and thyrotrophic hormones were found to affect liver weights.[7] Surprisingly, the effects of these preparations were found to be opposed to one another. Thyrotrophic hormone caused an absolute and relative increase in liver weights; growth hormone caused only a slight absolute increase which, however, was far less than the body weight increase produced by the hormone. Thus, when expressed in percent of body weight—which appears essential when comparing groups of widely varying body weights—growth hormone caused a relative decrease, thyrotrophic hormone

TABLE 1

*Effect of thyrotrophic, growth hormone and globulin fraction on liver weights of hypophysectomized rats**

NO. OF EXPT.	TYPE OF FRACTION	NO. OF RATS	BODY WEIGHT		LIVER WEIGHT			STATISTICAL SIGNIFICANCE OF DIFFERENCES
			Change	At autopsy	Absolute	Per 100 grams body weight	σ**	
			gm.	gm.	gm.	gm.		
1	Thyrotr. ($O_{VI}64C_{II}$)	9	+1	73	2.84	3.9	0.3	Incr. signif. (S_D = 0.14)
	Growth (IVF12CPP)	9	+13	93	2.96	3.2	0.3	Decr. signif. (S_D = 0.145)
	Globul. Fr. (PP_3)	9	+22	92	3.13	3.4	0.2	Not signif.
	Controls	9	0	70	2.50	3.6	0.2	
2	Thyrotr. ($O_{VIII}46BC$)	8	+5	68	3.67	5.4	0.6	Differ. highly signif. (S_D = 0.31)
	Growth (IVF121A)	6	+20	82	3.42	4.2	0.3	
	Globul. Fr. (ASXIII)	8	+17	82	3.82	4.7	0.4	
	Controls	5	−1	63	2.96	4.7	0.5	
3	Thyrotr. ($O_{VIII}46BC$)	10	+8	72	2.78	3.9	0.3	Incr. signif. (S_D = 0.14)
	Growth (IVF91A)	10	+20	86	2.67	3.1	0.3	Decr. highly signif. (S_D = 0.12)
	Globul. Fr. (AS XIII)	10	+27	92	3.27	3.5	0.2	Not signif.
	Controls	10	+6	69	2.45	3.6	0.2	

* Two-tenths milligram of each preparation administered daily for 10 days. The thyrotrophic preparations were kindly supplied by Jane Fraenkel-Conrat; they contained 30 to 50 chick units per milligram. The growth hormone and globulin fractions contained 20 to 50 growth units (hypophysectomized rats) per milligram.

** σ stands for the standard deviation from the mean of all liver weights within each group expressed in per cent of body substance.

an increase in liver weights compared to the controls. These findings were repeatedly confirmed (6 experiments, 200 rats) and statistical analysis of all the data has shown both the increase and the decrease to be highly significant (tables 1, 2).

[7] Not included in the tables are experiments with other pituitary hormones. Of these adrenocorticotrophic hormone, prepared and studied by C. H. Li, appeared to increase liver weights in preliminary experiments. Neither FSH, ICSH nor lactogenic hormone, the latter at various doses ranging from 0.2 mgm. to 5 mgm. daily, affected liver weights.

Globulin fractions[8] which contain growth, thyrotrophic, lactogenic and gonadotrophic hormones caused a proportional increase in liver and body weights, the liver: body weight ratio not differing from that of the controls (tables 1, 2). This indicates a "neutralization" of the two divergent tendencies. Neutralization also occurred when a mixture of purified growth and thyrotrophic hormones produced relative liver weights intermediate between those given by each hormone alone. In this experiment 0.08 mgm. was given daily for 15 days;

TABLE 2

*Summary of the liver weights of all rats treated with these pituitary preparations**

	NO. OF RATS	LIVER WEIGHT PER 100 GRAMS B.W.	DIFFERENCE BETWEEN EXPERIMENTAL AND CONTROL GROUP	
			S_D	Statistical significance
		grams		
Thyrotrophic hormone	58	4.42	0.13	Increase highly significant
Growth hormone	30	3.52	0.14	Decrease highly significant
Globulin fraction	33	3.95		No difference
Controls	44	3.95		

* Up to June, 1941 and since confirmed in several additional groups.

TABLE 3

Food intake, liver composition and oxygen consumption of rats treated with various preparations (expt. table 1, 3; table 4)

PREPARATION	FOOD INTAKE PER DAY PER RAT	LIVER COMPOSITION			OXYGEN CONSUMPTION
		Nitrogen	Fat	Water	
	grams	*per cent*	*per cent*	*per cent*	*l./m.²/24 hrs.*
Thyrotrophic H	5.6	3.2	2.8	73.7	159
Growth hormone	5.4	3.2	2.9	73.9	122
Globulin fraction	6.8	3.1		74.2	163
Controls	4.7	3.2	3.0	74.3	123
Thyroxin:					
0.025 mgm.		3.3	2.6	76.0	209
0.0075 mgm.	5.6	3.6	3.1	74.2	154
0.0025 mgm.		3.6	3.5	73.0	147
Controls	4.4	3.6	3.2	74.0	127

thyrotrophic hormone caused the livers of 8 rats to weigh 4.7 per cent ($\sigma = 0.2$), growth hormone (6 rats) 4.1 per cent ($\sigma = 0.2$), the mixture of both (0.16 mgm/day, 10 rats) 4.5 per cent ($\sigma = 0.3$) of the body.

Analyses[9] of nitrogen, fat, water and glycogen content of the livers of rats treated with the various fractions has revealed no differences in their composition (table 3). Thus the increase produced by thyrotrophic hormone must be regarded as a true increase in liver substance or growth of this organ.

[8] Prepared from beef anterior pituitaries according to 5.

[9] Kindly performed by V. V. Herring and W. La Salle.

Determinations of the food intake of rats treated with the various fractions has revealed both growth hormone and thyrotrophic hormone as causing a slight increase in the food intake, an effect which is far greater when the unfractionated globulin was given (table 3). This finding corresponds to expectation, in view of the differences in the oxygen consumption of these groups (which was also determined). Thus growth hormone treated rats need material only to build up body substance; thyrotrophic hormone treated rats need it as fuel in view of their increased metabolic rate; and those receiving cruder fractions need it for both growth and increased oxygen consumption. Thus differences in the effect of purified thyrotrophic and growth hormone on liver weights are not due to differences in food consumption.

II. Thyroxin (tables 3, 4). The finding that thyrotrophic hormone has a specific growth stimulating effect on the liver of hypophysectomized rats raised the question whether this effect was mediated by the thyroid and could be reproduced by the administration of thyroxin. Thyroxin at various levels

TABLE 4

Effect of thyroxin on liver weights of hypophysectomized rats

DAILY DOSE PER RAT	NO. OF RATS	BODY WEIGHT		LIVER WEIGHT			STATISTICAL SIG. OF DIFF. BETWEEN EXPER. AND CONTROLS
		Change	At autopsy	Absolute	Per 100 grams body weight	σ	
mgm.		*grams*	*grams*	*grams*	*grams*		
0.025	8	−4	65	3.22	5.0	0.54	S_D = 0.21, highly significant
0.0025	10	+4	73	2.86	3.9	0.3	Not significant
Controls	10	−2	65	2.44	3.8	0.3	
0.0075	9	+7	67	2.41	3.6	0.3	S_D = 0.155, highly significant
Controls	6	+1	63	1.94	3.1	0.3	

ranging from 0.0025 to 0.025 mgm. daily was therefore given to similar rats for 10 and 15 days. It was found that a dose of 0.0075 mgm. of thyroxin which, like 0.2 mgm. of thyrotrophic hormone, brought the oxygen consumption of the hypophysectomized rats back almost to normal levels, also led to an increase in liver weights which was similar to that produced by the pituitary hormone. Also the composition of these livers was unchanged, so that thyroxin must have caused a true increase in liver substance. A higher dose of thyroxin (0.025 mgm.) caused even more pronounced liver weight increase, in this case, however, associated with a slight relative decrease in nitrogen and fat content and increase in water. Also in these rats with their pronounced hyperthyroid state, however, the total amount of liver nitrogen in percent of body weight was increased above that of the controls. Thus it is evident that thyroxin causes increase in liver substance in hypophysectomized rats under our experimental conditions, similar to the effect observed by Sternheimer in his experiments. The effect of thyrotrophic hormone on liver weights can therefore be regarded as predominantly if not entirely mediated by the thyroid gland.

SUMMARY

1. Purified thyrotrophic hormone was found to cause absolute as well as relative increases in weight of the livers when administered for 10 or 15 days to young hypophysectomized rats.

2. Thyroxin administered under the same conditions also caused similar liver growth effects so that the effect of the thyrotrophic hormone may be attributed to its stimulation of the thyroid gland.

3. Purified growth hormone under the same conditions was found to cause only slight absolute increases representing significant relative decreases in the liver weights.

4. The effects of neither fraction were associated with changes in the composition of these livers, so that they must be regarded as actual changes in the amount of liver substance.

5. The effects on the liver are not due to differences in the food intake of the treated rats for both thyrotrophic and growth hormone caused a similar increase in food intake.

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EFFECT OF PURIFIED PITUITARY PREPARATIONS ON THE INSULIN CONTENT OF THE RAT'S PANCREAS[1]

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The existence in beef anterior pituitaries of a principle which increases the insulin content of the rat's pancreas has been well established by the work of Marks and Young (1, 2), as well as Soong (3). Doubt has been cast upon the physiological rôle of this principle in recent reports. Thus Haist and Best (4, 5) find that after the removal of the pituitary, rats show no lower pancreatic insulin content than normal control rats restricted to the same caloric intake; Griffiths and Young (6) also find no effect or a slight increase in pancreatic insulin content following hypophysectomy. These authors also report that in hypophysectomized rats no "pancreatrophic" effect could be produced by the administration of pituitary extracts which had pancreatic insulin increasing potency in normal rats. Attempts to identify the pancreatrophic factor with any of the established pituitary hormones have led Marks and Young to conclude (2) "that the pancreatrophic factor (which is probably not a hormone) is not identical with prolactin, nor with gonadotropic, thyrotropic or glycotropic substances." Their previous work (1) had differentiated it from the growth hormone.

Since considerable progress in the purification of various pituitary hormones has been recently achieved in this laboratory (see 7), it appeared indicated to study the effect of such preparations on the insulin content of rat pancreas.

METHODS. The experimental animals were generally 47 to 49 day old male rats of the Long-Evans strain. Rats, when hypophysectomized were usually operated upon when 26 to 28 days old; in one experiment adrenalectomized rats[2] were used as specified in the tables. The injections were performed intraperitoneally, $\frac{1}{2}$ or 1 cc. daily (except Sundays) for periods ranging from 10 to 42 days, but usually for 3 weeks. All animals were fasted before autopsy, usually for 5 hours and anesthetized with sodium amytal. To ensure complete removal of the diffuse pancreatic tissue, the entire mesenteries were stripped off the intestine. The mesenteries and pancreatic tissue of each group of rats (usually 5–10 rats) were pooled and minced. Insulin was extracted following the

[1] Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation, New York City, and Parke, Davis and Company, Detroit, Michigan. We wish to acknowledge assistance from the Works Projects Administration, Project no. OP-65-1-08, Unit A-5.

[2] We are indebted to Dr. E. Anderson for these rats.

procedure of Fisher (8). The crude powders thus obtained were dissolved in 0.85 per cent sodium chloride at pH 2–3; they were made up to such a concentration that the same given volume corresponded to 100 grams rat weight for all groups of one experiment, which were to be compared. These solutions were then injected into groups of 10 to 15 mice. Generally mice ranging from 17 to 22 grams were employed, animals of closely similar weights being used for each assay. They were fasted for 5 hours prior to the injection. The pancreas of treated and control rats were always assayed parallel and in most instances simultaneously also with standard insulin solutions. After injection, the mice were kept under observation for 90 minutes in an incubator (35–36°C). The number of mice in each group showing convulsions was noted.[3]

Each pancreatic extract was assayed at least twice and usually three to five times or in a total of 30 to 60 mice. The extracts could be kept frozen (at −10°C) for several weeks between tests without loss of potency.

TABLE 1

Insulin content of the pancreas of uninjected rats

TYPE OF RAT	AGE		NO. OF GROUPS OF RATS	INSULIN CONTENT (U./100 GRAMS RAT)	
	At autopsy	Post-operative		Average	Range
	days	*days*			
Normal male	60–80		9	0.54	0.26–0.97
Normal female	51–86		5	0.84	0.44–1.3
Hypophysectomized male	70–89	17–21	2	0.7	0.41–1.0
Hypophysectomized female	44–72	17–24	6	0.82	0.56–1.3
Adrenalectomized male	70	18	1	1.47	

The potency of unknown pancreatic extracts was always calculated and expressed in units of insulin per 100 grams rat. From those cases where one solution has been assayed 5 or more times it was possible to calculate the standard deviation which was found not to exceed ±17 per cent. Thus it would appear that differences exceeding 34 per cent between the pancreatic insulin content of two groups of rats can be regarded as significant. In agreement with Marks and Young (2) we are not inclined to attribute much significance to differences of less than 50 per cent unless confirmed by repetition of the experiment.

A short discussion of the pancreatic insulin content of uninjected control rats is indicated. Table 1 summarizes all such assays performed in the course of one year. Although great variations were noted at different times in the

[3] They were immediately removed from the incubator and could usually be saved by the intraperitoneal administration of approximately 0.5 cc. 20 per cent glucose. The same mice were used repeatedly with rest periods of at least 1 week and were not found to become less responsive to standard insulin doses, when used 4 times in this manner, as long as their weights did not exceed 24 grams.

same type of rat,[4] the higher average insulin content in the normal females as compared with the males appears worthy of attention; no such difference was noted between hypophysectomized rats of the two sexes. In agreement with findings of other investigators (4–6) hypophysectomy per se did not significantly affect insulin levels. In 2 experiments where hypophysectomized and normal control rats[5] were compared directly and simultaneously, the pancreas of the hypophysectomized rat was found to contain 53 per cent more in one case and 18 per cent less insulin than the controls in the other. A similar comparison of adrenalectomized and normal rats in a single experiment indicated that the

TABLE 2

Effect of growth hormone on pancreatic insulin content*

EXPERIMENTAL RATS							CHANGE IN PANCREATIC INSULIN CONTENT	
Type	Age at autopsy	P.O. age at autopsy	Injection period	Daily dose	Number			
					Injected	Control		
	days	*days*	*days*	*mgm.*			*per cent*	
Normal male	68–70		21	1.0	7	7	−36	
					4	5	−45	
					6	9	−70	
					5	10	−68	−40
					5	10	−32	
					6	7	−39	
					6	6	+9	
				2.0	6	7	−39	
				3.0–9.0**	2	5	−24	
Adrenalectomized male	70	18	16	1.0	4	5	−37	
Hypophysectomized male	70–77	17	17	2.0	2	5	+15	
Hypophysectomized female	47–56	23	16	1.0	3	4	−15‡	

* 4 different preparations, containing 20–40 hypophysectomized rat units/mgm.

** First week: 3.0 mgm. daily; second week: 6.0 mgm. daily; third week: 9.0 mgm. daily.

‡ Compared to the effect of adrenocorticotrophic hormone, marked similarly, not to the control group as usual (lost by accident).

pancreas of the adrenalectomized rat contained 123 per cent more insulin than of the unoperated rat.

RESULTS. Three pituitary fractions were found to affect the insulin content of the rat's pancreas: lactogenic hormone and adrenocorticotrophic preparations

[4] In some of the earlier experiments the insufficient use of insulin standard solutions may be responsible for inaccurate absolute values (which does not invalidate comparative data of the insulin content of various groups of rats, assayed simultaneously).

[5] Receiving unlimited amounts of a different diet.

TABLE 3

Effect of lactogenic hormone on pancreatic insulin content*

EXPERIMENTAL RATS								
Type	Age at autopsy	P.O. age at autopsy	Injection period	Daily dose	Number		CHANGE IN PANCREATIC INSULIN CONTENT	
					Injected	Control		
	days	*days*	*days*	*mgm.*			*per cent*	
Normal male	68–70		21	1.0	8	8	+160	
					4	5	−31	+76
					6	6	+100	
				0.33	5	5	−24	
Normal female	68		42	0.5–2.0**	4	4	+50	
	51		30	2.0	6	6	0	
Hypophysectomized female	50	24	17	1.0	5	5	+110	
					4	7	+79	+95
	43	17	10	1.0	5	6	0	
				0.2	8	6	−16	

* Nine different preparations were employed, all containing 25–30 IU per mgm.
** Increasing 0.5 mgm. every tenth day.

TABLE 4

Effect of adrenocorticotrophic hormone on pancreatic insulin content

EXPERIMENTAL RATS								
Type	Age at autopsy	P.O. age at autopsy	Injection period	Daily dose	Number		CHANGE IN PANCREATIC INSULIN CONTENT	
					Injected	Control		
	days	*days*	*days*	*mgm.*			*per cent*	
Normal male	68–70		21	1.0	6	6	+25*	
				2.0	9	9	+55	+62
					8	7	+69	
Hypophysectomized male	70–90	21	21	5.0	5	6	+260	
		17	17	5.0	5	8	+290	+275
		10	10	5.0	6	3	0	
		17	17	1.0	5	5	+24	
Hypophysectomized female	50–55	23	15	1.0	10	4	+20	
	55	28	10	1.0	9	8	+28	
	43	18	10	1.0	7	7	+43	
	50–55	28	20	1.0	6	4	+18**	

* This preparation (which was also used in hypophysectomized rats, see last experiment on this table), is considerably more active and freer from lactogenic hormone than all others. It caused (at 1 mgm. daily dose) adrenal weight increases of 60 per cent and stunted growth. Two milligrams daily of the other preparations caused adrenal weight increases of 43 per cent and 32 per cent and no significant growth inhibition.

** +18 per cent, compared to the effect of growth hormone (see table 2), not to control group.

increased it while growth hormone decreased it (tables 2 to 4).[6] In interpreting these findings it should be remembered that all lactogenic hormone preparations used contained 25 to 30 IU per mgm; preparations of such activity have been shown to be chemically pure, as judged by electrophoretic, ultracentrifuge and solubility studies (9). The growth hormone used for these studied had been treated with cysteine and therefore contained no more than traces of lactogenic, thyrotrophic or gonadotrophic hormones (10); it did contain from 5 to 10 per cent adrenocorticotrophic hormone. Of the adrenocorticotrophic preparations all but one (as indicated in the table) contained considerable contamination with the lactogenic hormone (possibly as high as 25 per cent) but no more than traces of other target organ hormones.[7]

TABLE 5

Effect of various preparations and conditions on pancreatic insulin content

(In normal male rats, 60–70 days at autopsy, injected for 21 days)

TYPE OF TREATMENT	DAILY DOSE	NO. OF RATS		CHANGE IN PANCREATIC INSULIN CONTENT
		Injected	Control	
				per cent
A. Crude beef anterior pituitary extract	10 mgm.*	10	10	−11
	53 mgm.**	8	8	+55
B. Globulin fraction‡	2 mgm.	7	7	+8
C. Thyrotrophic hormone†	2 mgm.	6	7	−8
D. Adrenal cortex extract (Upjohn)	1 cc.	6	9	−33
E. High carbohydrate diet§		7	7	+16

* Equivalent to 220 mgm. fresh gland daily.

** Equivalent to 1000 mgm. fresh gland daily.

‡ Prepared from alkaline extract of beef anterior pituitaries; contains growth, lactogenic, thyrotrophic, adrenocorticotrophic hormone and ICSH.

§ High carbohydrate diet: 68 per cent cornstarch, 18 per cent casein, 6 per cent brewer's yeast, 1 per cent cod liver oil, 4 per cent salts, 3 per cent lard. Also during the last week ½ cc. daily Galen B and 20γ riboflavin, also twice daily 5 cc. 40 per cent glucose by stomach tube. Control diet: 40 per cent starch, 30 per cent casein, 10 per cent yeast, 15 per cent lard, otherwise as above (no glucose by stomach tube).

† Prepared from beef pituitaries according to 11, contains 30 chick units per milligram.

Unfractionated alkaline extracts of beef anterior pituitaries had a slight pancreatrophic effect only upon administration in excessive doses. A globulin fraction prepared from such extracts had no effect on the pancreas at the level tested; neither had a purified thyrotrophic preparation (11) (table 5).

Discussion. The finding that two purified pituitary fractions, lactogenic and growth hormone, had opposite effects on the pancreatic insulin of rats was at first surprising. It then became apparent, however, that this fact afforded a

[6] No evidence for "hyperinsulinism" was found in rats with increased pancreatic insulin; blood sugar determination of such rats gave values which were slightly above those of the controls, the differences being statistically not significant.

[7] Part of the growth hormone preparations were kindly supplied by Dr. W. Marx; part of the lactogenic and adrenocorticotrophic preparations by Drs. W. R. Lyons and C. H. Li.

possible explanation for the inefficacy of crude alkaline extracts of beef anterior pituitaries and the globulin fraction derived therefrom to affect the pancreatic insulin content.[8] The existence of these opposing influences of the pituitary on the pancreas may also explain why neither hypophysectomy (4, 6) nor the implantation of rat pituitaries into hypophysectomized rats (12) produces any change in the insulin content of the pancreas. In short, our finding of the antagonistic action of 2 pituitary fractions explains why removal of both (hypophysectomy) or administration of both factors in balanced amounts (crude extracts or implants) may produce no effect on the pancreatic insulin content.

Growth hormone was found to decrease the insulin content of the pancreas of normal rats, treated for 3 weeks (1 or 2 mgm. daily) (table 2). There is little doubt that this action is associated with the growth promoting activity of purified preparations. The only known hormonal contaminant of such growth preparations is the adrenocorticotrophic hormone and this hormone exerts the opposite effect on pancreatic insulin. It must also be noted that growth hormone appears to be effective in decreasing the pancreatic insulin of adrenalectomized rats; in hypophysectomized rats it has not shown any effect, a question which will be further investigated. Attempts to reduce the pancreatic insulin to a minimum and possibly to produce diabetes by administration of excessive doses of growth hormone have not been successful (table 2).

Lactogenic hormone has produced pronounced pancreatrophic effect in five of seven experiments in normal and hypophysectomized rats when 1 mgm. daily was administered for at least 17 days[9] (table 3). Shorter injection period (10 days) or lower doses of the hormone produced no effects. Since the pronounced pancreatrophic effects were obtained with several different hormone samples prepared by different methods, and biologically and physico-chemically close to pure, it appears established that the lactogenic hormone, directly or indirectly exerts pancreatrophic activity in normal and hypophysectomized rats.[10]

Table 4 summarizes the results of the administration of adrenocorticotrophic preparations on pancreatic insulin content of normal and hypophysectomized rats. It will be noted that increases were regularly obtained. These were marked only when very high doses were used (5 mgm. daily), while 1 mgm. daily of the most active preparation[11] had no significant pancreatrophic effect. All adrenocorticotrophic preparations with the exception of the latter, contained

[8] Marks and Young (1) find considerable pancreatrophic activity in unfractionated beef pituitaries but not in horse pituitaries. In discussing the lack of pancreatrophic activity of horse pituitaries they refer to the possibility of its action being masked by some counteracting factor.

[9] It is not yet understood why in two experiments performed under the same conditions no increase in pancreatic insulin occurred, just as growth hormone was ineffective in one of nine experiments. As far as could be ascertained, this was not due to deterioration of the preparations and therefore has to be attributed to biological variability.

[10] Marks and Young found a less purified prolactin to be strongly pancreatrophic but arrived at the conclusion that the 2 factors were not identical.

[11] Supplied by C. H. Li.

10 per cent to 25 per cent lactogenic hormone which has given pronounced pancreatrophic effects at a daily dose of 1 mgm; the interpretation therefore suggests itself that the observed effects may be due to the lactogenic contamination, rather than to the adrenocorticotrophic hormone proper. Further assays of more purified preparations will enable us to settle this question.

When the first pancreatrophic effects were obtained with adrenocorticotrophic hormone, it was attempted to duplicate its effect by the administration of adrenal cortical extract, also by feeding a high carbohydrate diet. These attempts were not successful (table 5). The high pancreatic insulin content of adrenalectomized rats (maintained on salt) also negates the importance of cortical hormones for maintenance of pancreatic insulin. Primary regulation of the pancreas by the adrenals has also been ruled out by Haist's recent findings (13) that neither the administration of cortical extracts (at higher levels than in our experiments) nor adrenalectomy showed any effect on pancreatic insulin. All factors therefore support the interpretation that positive pancreatrophic effects of crude adrenocorticotrophic preparations are due to contamination with lactogenic hormone, rather than to their intrinsic effect on the adrenals.

SUMMARY

The effect of various hormones of the anterior pituitary on the insulin content of rat pancreas has been investigated. Pure lactogenic hormone was found to increase the insulin, growth hormone to decrease it. The insulin increasing action of crude adrenocorticotrophic preparations was attributed to contamination with lactogenic hormone.

Lactogenic hormone showed pancreatrophic activity in both normal and hypophysectomized rats, while the opposite action of the growth hormone could be demonstrated in normal and adrenalectomized but not in hypophysectomized rats.

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EFFECT OF GRAVITY ON THE BLOOD PRESSURE OF THE DOG[1]

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The effect of posture on the blood pressure in man has been the subject of considerable experimentation. The change from the recumbent to the standing position suddenly shifts a column of blood, the length of the body, from a horizontal to a vertical position where it exerts a hydrostatic pressure which it did not exert previously. This flooding of the capillary reservoirs in the dependent parts is usually accompanied by a temporary drop in the systolic and a slight rise in the diastolic pressure. Within a few seconds, however, compensation sets in, principally in the form of a generalized vasoconstriction, the systolic pressure recovers a large percentage of the drop and the diastolic pressure remains elevated (1). Attempts to elucidate the mechanism responsible for the compensation have yielded conflicting results. The suggestion that the vasoconstriction was mediated reflexly via the pressor receptor (carotid sinus and aortic) nerves (2) has been challenged by Erdholm (3) and Conklin and Dewey (4) who failed to observe a decrease in compensatory ability of their experimental animals after elimination of these pathways. Erdholm suggests that this variation in experimental results may be due to the animal used and to the fact that several workers have used anesthetics, such as morphine, which depress the respiratory center and so may interfere with the respiratory pump. He further believes that previous investigators failed to appreciate that there was fatigue of the compensatory mechanism and that repeated tiltings produce an increasing blood pressure fall even in the intact animal. In view of these divergent findings it seems appropriate to report the results of experiments which were performed on dogs but which are not, we believe, subject to the criticism which Erdholm had levied against previous work. These results reaffirm the importance of the pressor receptor nerves in the compensatory reaction of the dog to gravity. They also indicate that compensation in the dog is quite effective and that it is usually as good as in man.

PROCEDURE. One hundred and thirty experiments have been performed on 36 dogs. Eighteen of these were lightly anesthetized with chloralose (40–80 mgm. per K.B.W.) and a like number with sodium barbital (180–250 mgm. per K.B.W.). They were placed in an animal trough which was rotated about a

[1] Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

transverse horizontal axis, the position of which was adjusted in each case to coincide with the axis of the cannula used for recording blood pressure. The latter was recorded from the carotid or the femoral artery on a moderately fast kymograph by a calibrated membrane manometer using chlorazol fast pink as anticoagulant. In a number of experiments simultaneous determinations of the mean intracarotid and intrafemoral pressures were made by puncture of these arteries according to the method of Dameshek and Loman (5). In these experiments the axis of rotation was adjusted to approximate that of the heart. The animal was prevented from slipping when in the upright (75°) position by tying the mouth securely around a bit fixed to the animal board. In the earlier experiments respiration was recorded by means of a pneumograph fastened about the chest; in later experiments by a small T-tube inserted directly into the thoracic cavity through a stab wound.

The usual procedure was to take a short control tracing in the horizontal position after which the animal was tilted to an angle of 75°, feet down, and kept in this position for periods varying from 1 to 20 minutes before being returned to the horizontal. The tilting usually took about 2 seconds. The subsequent conduct of the experiments was varied. In some instances observations were made during repeated tiltings of the intact animal, in others the effects of tilting were observed after the vagi were cut or the carotid sinuses denervated by stripping the internal carotid artery and painting the region with phenol. In the latter experiments the tiltings were usually repeated subsequent to complete interruption of the pressor receptor mechanism.

Results and discussion. The changes in blood pressure produced by tilting to the F.D. (feet down) position varied considerably, due to the practical impossibility of achieving and maintaining the same level of anesthesia in different animals, as well as to variations in their compensatory respiratory and vasomotor mechanisms. Typical results are shown in figure 1. Simultaneous measurements of the intrafemoral and intracarotid pressure by direct puncture showed that the mean pressures in these arteries were practically identical when the animals were in the horizontal position. Immediately on changing the position the intrafemoral pressure in some cases increased to a level approximating the added hydrostatic component, calculated as the pressure of a column of blood from the fourth interspace (heart level in the F.D. position) to the point of cannulation or puncture. This level was seldom maintained for more than a few seconds, being followed by a drop of varying degree and a secondary rise, so that while the pressure remained above the control horizontal level throughout the F.D. position it was still less than the sum of the hydrostatic and hydrodynamic factors. In other experiments, the intrafemoral pressure fell sharply on tilting and then rose gradually to levels above the control horizontal values. In 15 animals the average fall in intrafemoral pressure within 10 seconds after tilting was 9 mm. Hg (6.3 per cent) and the average levels during and at the end of the F.D. period were 12 and 9 mm. Hg (7.9 and 6.3 per cent) respectively above the pre-tilting value. Since the average hydrostatic component was 22

mm. Hg (15 per cent), these results indicate a decrease in the hydrodynamic component in the majority of the experiments.

The changes in intracarotid pressure follow a similar pattern, the change in position being followed by an immediate sharp drop to values which in most experiments exceeded the difference between the hydrodynamic and hydrostatic factors (since above heart level gravity opposes the hydrodynamic factor). In the 21 animals in which the intracarotid pressure was recorded there was an average fall of 39 mm. Hg (27.5 per cent) within 10 seconds after the animal was tilted, an average decrease of 37 mm. Hg (26.5 per cent) during the F.D. posi-

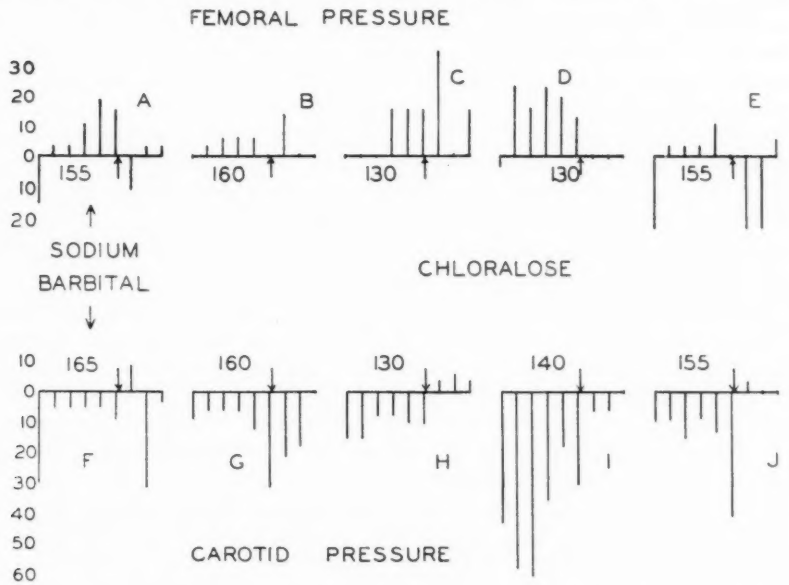

Fig. 1. Blood pressure changes in the F.D. position. The first point plotted is the per cent change from the horizontal level 10 seconds after tilting. The successive points are similar values for 20, 30, 60 seconds, 5 and 10 minutes after tilting and 10, 20 and 60 seconds after return to the horizontal position. The figures given with each graph are the control horizontal values. The arrow denotes return to the horizontal position.

tion and of 44 mm. Hg (31.2 per cent) at the end. The average hydrostatic component amounted to 13 mm. Hg (9 per cent) in these experiments. Simultaneous measurement of the intracarotid and intrafemoral pressures showed that the maximum fall in the former usually coincided with the greatest rise in the latter and that the pressure curves during the F.D. period showed more or less parallel changes. In a large majority of animals the blood pressure reached a steady state within 2 minutes, showing only slight further change as the F.D. period was prolonged. On return to the horizontal position there was usually an immediate rise in the blood pressure (carotid and femoral) amounting to

values of 50 per cent above the control level in some instances followed in a few seconds by a dip below and then a gradual return to the original level.

These changes in intrafemoral and intracarotid pressure are qualitatively and quantitatively similar to those reported by Loman, Dameshek, Myerson and Goldman (6) who determined the pressure changes in man by direct puncture. Our findings contradict the usual inference made on the basis of Leonard Hill's early work (7) that compensation to gravity in the dog is poorer than in man. The failure of the intracarotid and intrafemoral pressures to reflect absolutely their respective hydrostatic components suggests that the assumption of the upright position is accompanied by a generalized passive dilatation, probably involving arterioles, capillaries and veins which results in a diminished venous return and consequent lowered cardiac output and arterial pressure. The decreased arterial pressure evokes a compensatory arteriolar vasoconstriction which, in the dog as well as in man (8) is seldom, if ever, complete but is often adequate to prevent serious cardiovascular embarrassment even when the animal is kept in the F.D. position for periods of 4 to 6 hours.

No significant relationship was found between the initial level of blood pressure and the average or final change during the F.D. period. Likewise, no significant differences in response were observed between the results when chloralose or sodium barbital was used as the anesthetic.

Figure 2 shows the results of successive tilting in 3 representative experiments. There was no evidence of fatigue of the compensating mechanisms in the intact animal in spite of the relatively long periods in which the animals were kept in the F.D. position, except in those instances where the anesthesia was very deep or the condition of the animal was poor as judged by a low initial pressure and poor compensation on the first tilt. In several instances (fig. 2 B) compensation actually improved with successive tilts.

Figure 3 shows typical results of tilting animals deprived of their pressor receptor nerves. They leave no question as to the primary importance of these mechanisms in the response to gravity. Denervation of the carotid sinuses or vagotomy resulted in every case in a diminished ability of the animal to compensate for the F.D. position. This is strikingly shown by the changes in intrafemoral pressure (fig. 3 C) when the hydrostatic component completely disappeared after vagotomy. In several experiments the elimination of only one set of pressor receptors produced questionable differences on the first tilt. If, however, the tilt was repeated, there was definite evidence of fatigue and failure of the remaining mechanism to maintain adequate compensation (fig. 3 B). Elimination of the two pressor mechanisms resulted in many cases in a rapid drop in pressure to shock levels from which recovery was slight (fig. 3 C).

The removal of the pressor receptor nerves not only handicaps the compensation of the animal while in the F.D. position but also significantly alters the character of the recovery on return to the horizontal position. As previously indicated, the sudden shifting of blood back to the heart when the intact animal is returned to the horizontal is accompanied by a marked rise in blood pressure. This is followed by a dip below normal for a few seconds before return of the

pressure to the control level. After elimination of the pressor receptor mechanisms, however, return to the horizontal position is followed by a much smaller and more gradual rise in pressure which seldom achieves the control level and never shows the characteristic dip.

Erdholm and McDowell (9) contended that the response to gravity is unaffected by the loss of the buffer nerves providing care is exercised in avoiding loss of CO_2 from over-ventilation which reduces the activity of the vasomotor

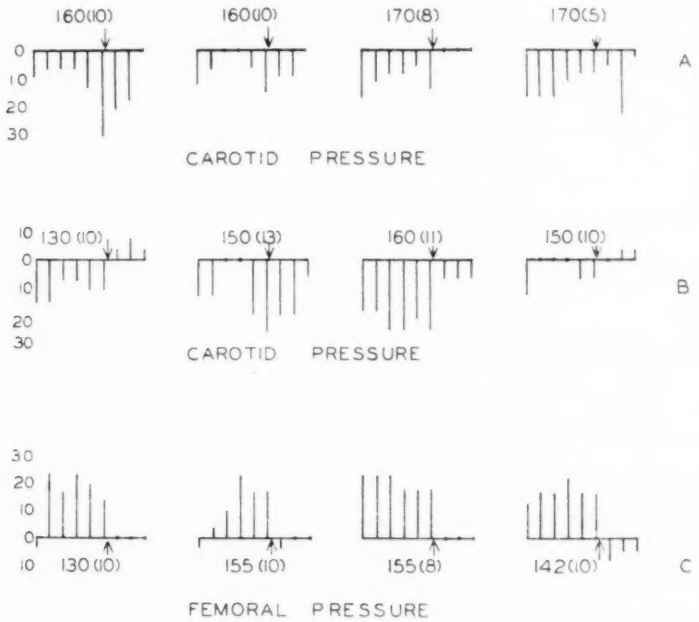

Fig. 2. Effects of successive tilts on the blood pressure. The changes are given as percentages of the horizontal pressure which is given for each case. The points plotted are the respective observations at 10, 20, 30 and 60 minutes after tilting and at the middle and end of the F.D. period and 10, 20 and 60 seconds after return to the horizontal position, the time of which is indicated by the arrows. The figures in parentheses denote the length of the F.D. period.

centers. Our experiments indicate, however, that compensation is handicapped even in those instances when respiration is not altered appreciably after loss of the pressor receptor nerves. To test this point further, three sets of experiments were performed in which animals were made to breathe mixtures of 7 to 8 per cent CO_2 in air from large Douglas bags and placed in the F.D. position before and after successive elimination of the carotid sinus and vagus nerves. No significant differences were observed in the responses after denervation when CO_2 was breathed as compared to control observations on the same animals breathing room air.

The changes in pulse and respiration rate were variable. In most experiments tilting was followed by an immediate rise in the pulse rate which was maintained or increased during the F.D. period. In some cases, however, there was no change or a slight drop for several minutes after tilting, the rate rising during the latter part of the F.D. period. Denervation was usually followed by a marked increase in pulse rate to a point which precluded accurate counting but the rate did not appear to change significantly when the animal was in the

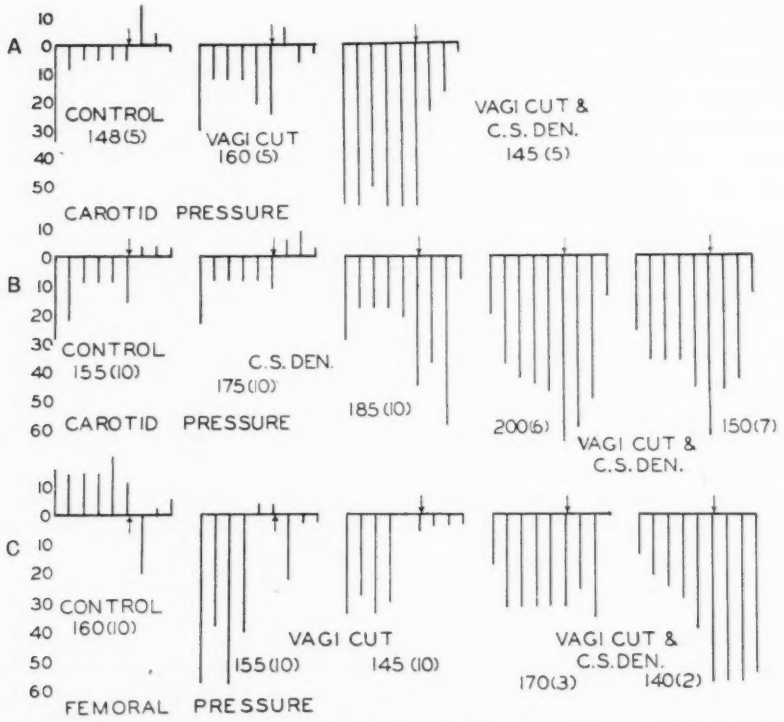

Fig. 3. Effects of removal of pressor receptor system. Explanation of chart same as in figure 2.

F.D. position. Respiration usually became slower and deeper immediately following tilting but increased progressively during the F.D. period to values above those obtaining in the control period. In several experiments, however, rapid shallow breathing obtained throughout the F.D. period. After denervation the respiration usually remained slower and deeper during the F.D. period, and in some cases became periodic or stopped completely after relatively short periods (2 to 3 min.) in the upright position.

The changes in respiration following denervation suggest that the carotid sinus and aortic nerves are of importance in the response to gravity not only be-

cause of their pressor functions but because of their rôle in the response to lowered oxygen tension. The F.D. position tends to produce a greater or less degree of hypoxia (10) which, in turn, causes an increase in respiration and a secondary rise in blood pressure by action of the peripheral chemoreceptor zones rather than on the respiratory and vasomotor centers themselves (11). Thus much of the variation in the blood pressure responses in the same and in different animals can be correlated with differences in the character of the respiratory changes. In those experiments in which the respiratory patterns during successive F.D. periods were similar, the blood pressure changes showed little difference; when respiration changed considerably the blood pressure responses varied accordingly. Tilting after elimination of the two sets of pressor receptor nerves was followed in the majority of cases by periodic or complete cessation of respiration and, as previously indicated, by a virtual vasomotor collapse. In these experiments where the respiration stopped and the blood pressure was at shock level during the F.D. period, return of the animal to the horizontal position was followed by a recovery of the blood pressure to the normal level, but respiration failed to begin again.

SUMMARY

1. Tilting of anesthetized dogs from the horizontal to the upright, feet down (F.D.) position is accompanied usually by a sharp drop in intrafemoral and intracarotid blood pressure followed within 10 seconds by a compensatory rise of varying degree. While there is seldom complete compensation for the effects of gravity, there is usually an amount adequate to prevent serious cardiovascular embarrassment even when the animal is maintained in the F.D. position for several hours.

2. Repeated tilting does not seem to fatigue the compensating mechanisms except in those instances where the anesthesia is very deep or the condition of the animal is poor as judged by a low initial pressure and poor compensation on the first tilt.

3. Denervation of the carotid sinuses or cutting the vagi, thus eliminating the two aortic nerves, uniformly diminishes the animal's ability to compensate for gravity. The secondary rise in pressure in the F.D. period as well as the rise after the return to the horizontal position are smaller. In some cases compensation fails during the F.D. position. Elimination of both pressor receptor mechanisms usually results in complete absence of compensation and in many cases in a drop in pressure to shock levels from which recovery is slight.

4. The carotid sinus and aortic nerves are of importance in the response to gravity not only because of their pressor functions but because of their rôle in the response to lowered oxygen tension usually present when the animal is in the F.D. position.

I am indebted to Messrs. A. J. Carroll, Jr., C. A. Patterson, S. B. Crepea, W. D. Davis, Jr. and W. J. Trautman, Jr. who at various times assisted in the experiments.

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THYROID AND PARATHYROID HORMONE EFFECTS ON CALCIUM AND PHOSPHORUS METABOLISM

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The heavy excretion of calcium and the development of osteoporosis (1) in cases of hyperthyroidism has led to much speculation concerning the mode of action of the thyroid hormone on calcium metabolism. In view of the known effect of the parathyroid hormone on this metabolism, it is pertinent to compare experimentally the effect of these two hormones in this respect. Since active preparations of both glands are available, it is possible to observe the effect of these compounds both singly and in combination upon normal animals and upon those lacking thyroid and parathyroid glands.

If the effect of an excess of thyroid hormone upon calcium and phosphorus metabolism is not due to a stimulating action on the secretory activity of the parathyroid gland, the administration of active thyroid preparations, and of parathyroid hormone, to a normal animal might be expected to produce different metabolic responses. These experiments were performed in an attempt to shed light on this question. Experiments were also performed on thyroparathyroidectomized animals to show the action of the thyroid hormone on the calcium and phosphorus metabolism in the absence of the parathyroid hormone.

EXPERIMENTAL. The experimental animals were young male dogs weighing about 15 kgm. and in good health. Young animals were used because of their marked response to parathyroid hormone and their adaptability to a metabolism routine.

Animals were kept in metal metabolism cages throughout the experimental periods.

In experiment 1 the animal was given a diet of dog chow, starch and beef suet calculated to satisfy energy requirements and to contain only one per cent calcium. In experiments 2, 3, and 4 no food was given during the experimental period. Fresh water was provided ad libitum through all the experimental periods.

Experiments 1 and 2, carried out on different dogs, were divided into four consecutive periods. The first period was preparatory. The animals were placed in metabolism cages and maintained under experimental conditions for three days in order to obtain an approximately basal state. The second period was that of induced hyperthyroidism. This was produced in the animals by the administration by mouth of 1 gram/kilogram/day of desiccated thyroid (Ar-

mour) for three successive days. This was sufficient to produce and maintain a condition of marked hyperthyroidism throughout the experimental period, as evidenced by tachycardia and increased creatinine excretion. In the third period, a hyperparathyroid state was superimposed upon the condition of hyperthyroidism. This was accomplished, after a lapse of one day from the day of the last thyroid administration, by the subcutaneous injection of 20 units of parathyroid hormone per kilogram of body weight. This period consisted of only one day. The fourth and final period was a second control period representing the time required by the animals to return to the basal state.

Experiments 3 and 4 were duplicate experiments carried out on the dogs used in experiments 1 and 2, after they had been thyroparathyroidectomized. Each experiment consisted of two consecutive periods. The first was preparatory. The animals were placed in metabolism cages and maintained under experimental conditions until they had reached a basal state. At the beginning of the second period, the animals were thyroparathyroidectomized. Following the operation, hyperthyroidism was produced by feeding the animals 1 gram/kilogram/day of thyroid for a period of three days. In experiment 3 the thyroid administration was begun on the day of operation while in experiment 4 it was not given until one day following operation.

Throughout all periods of all four experiments collections of both blood and urine were made at least once a day. In the third period of experiments 1 and 2 samples were taken at one, four and twelve hour intervals. At the time of collection residual bladder urine was withdrawn by catheterization and the bladder washed with sterile, isotonic saline until the washings were clear. This material was added to the urine collected from the metabolism cage during this period, and the entire sample filtered and then thoroughly mixed to insure proper sampling. Care was taken to avoid fecal contamination. The completeness of each urine collection was checked by determining the creatinine output in each sample.

Immediately after catheterization, a blood sample was withdrawn by jugular puncture. The whole blood was allowed to clot, was centrifuged, and the serum taken for analysis.

Each urine sample was analyzed for inorganic phosphate, total calcium, and creatinine. Calcium and inorganic phosphate determinations were made on the serum, and except for the first experiment, alkaline phosphatase values were obtained for each serum sample.

Inorganic phosphate was determined by the method of Fiske and SubbaRow (2); calcium by that of Fiske and Logan (3); and phosphatase by that of Bodansky (4).

Results. The data obtained in the experiments are presented graphically in figures 1 to 4. The metabolism data are given in figures 1 and 3, the blood data in figures 2 and 4.

A. Thyroid administration to normal dogs. In experiments 1 and 2 the excretion of urinary calcium was increased tenfold on the second day of the administration of thyroid (figs. 1 and 3). Some increase in calcium excretion

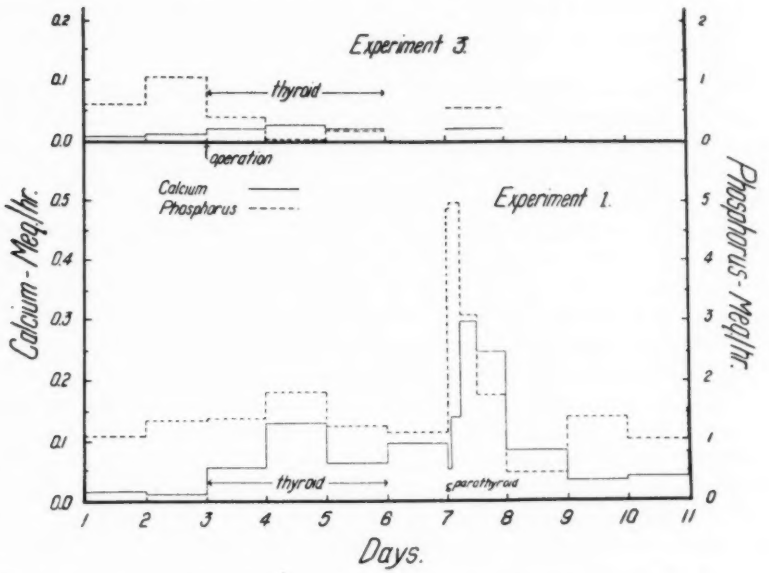

Fig. 1. Urinary excretion of calcium and phosphorus in experiments 1 and 3.

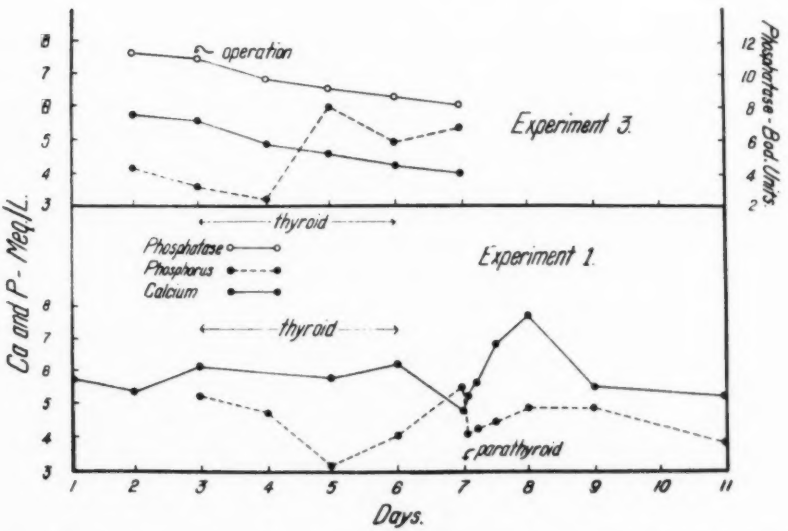

Fig. 2. Values for calcium, phosphorus, and phosphatase in the blood serum in experiments 1 and 3.

appeared on the first day of thyroid feeding. The increase in calcium excretion was accompanied by no marked change in the level of serum calcium (figs. 2 and 4).

The phosphorus excretion in the urine showed no marked or consistent alterations following thyroid administration; nor were the levels of serum inorganic phosphate affected. Phosphatase values of the serum showed no changes.

B. Parathyroid hormone administered to hyperthyroid dogs. The charts of experiments 1 and 2 show that the administration of parathyroid hormone to these hyperthyroid dogs produced the response known to be characteristic of this hormone (5). There was an immediate increase in phosphorus excretion, being most marked in the first hour after injection of the hormone. The serum phosphorus fell slightly following the injection of the parathyroid hormone.

Phosphatase values followed in experiment 2 remained essentially unchanged during this response to parathyroid preparations.

C. Thyroid administration to thyroparathyroidectomized dogs. After a lapse of two weeks, and following another control period, the thyroid and parathyroid glands were removed from dogs 1 and 2. There was a fall in phosphate excretion and in the concentration of serum calcium.

On the fifth post-operative day dog 1 developed tetany from which it spontaneously recovered and subsequently survived several months. Dog 2 died in tetany on the fourth post-operative day.

The phosphorus excretion, in both instances, was less in the post-operative period of thyroid administration than it was previous to both thyroid feeding and operation. In the experiment on dog 2 the phosphorus excretion was virtually zero.

The serum calcium dropped steadily during the post-operative period in spite of the thyroid administration. In both cases, the values reached a minimum of 4 meq. per liter. The administration of thyroid to these thyroparathyroidectomized dogs results in calcium excretions much less than those found in the same dogs treated similarly before the thyroid and parathyroid glands were removed.

The serum phosphorus in experiment 3 rose above 5 meq. per liter on the second post-operative day, and remained at that level. In experiment 4, also, there was a slight rise in serum phosphorus on the second post-operative day, but it quickly returned to preoperative levels.

The alkaline phosphatase values showed an increase of 2 and 5 units respectively by the fourth post-operative day.

Discussion. The data of these experiments demonstrate that an excess of the thyroid hormone will affect calcium metabolism in the normal dog. Whether it does so as the result of its direct action on calcium metabolism or indirectly through the parathyroid hormone has not been definitely answered by these experiments. There may be some question as to the possibility of a hyperthyroid diuresis having caused the increase in calcium output. The fact that, in our experiments, there was no relation between total urine volume and total calcium output indicates that this effect is not important. Under the influence

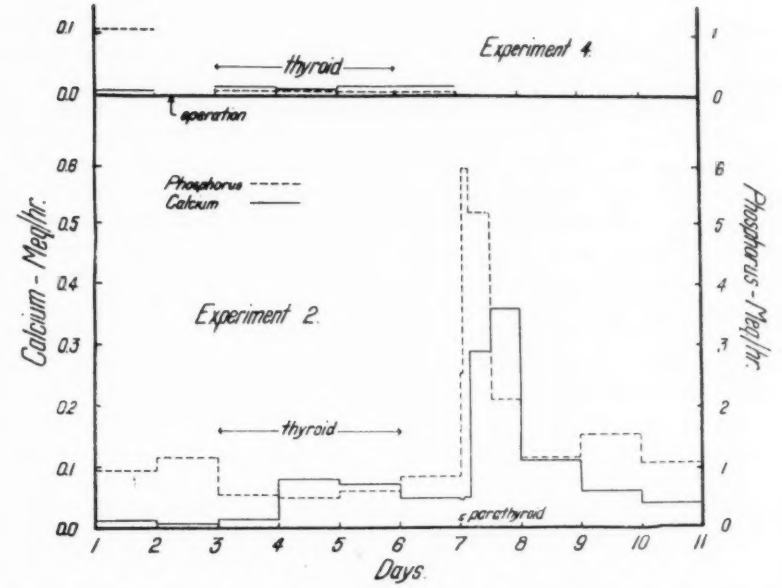

Fig. 3. Urinary excretion of calcium and phosphorus in experiments 2 and 4.

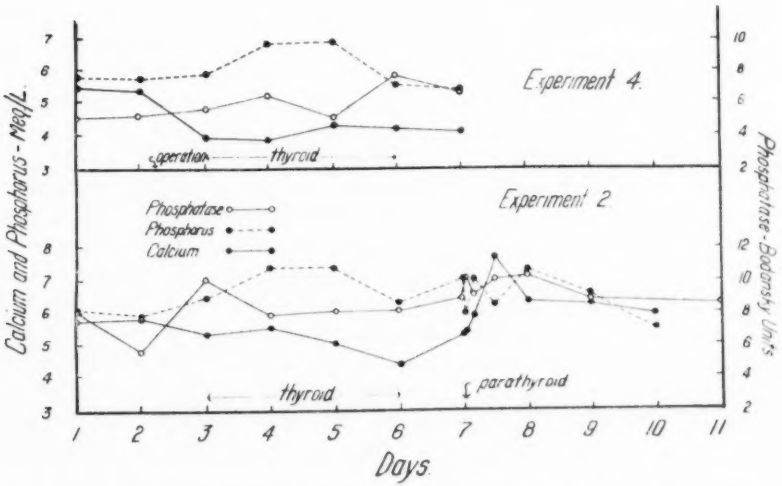

Fig. 4. Values for calcium, phosphorus and phosphatase in the blood serum in experiments 2 and 4.

of the thyroid hormone, the calcium output was approximately ten times its former value, while the total urine output was only about 2.8 times the normal volume. Nor is this increase due to neutralization of acid metabolites whose production has increased because of the higher metabolic rate (6).

The data show that the pattern of response to the thyroid hormone is different from that produced by the parathyroid hormone. While the thyroid had no consistent effect on phosphorus metabolism, the parathyroid hormone caused an immediate rise in urinary phosphorus and a simultaneous drop in serum phosphorus. Further, thyroid administration produced a gradual rise in calcium excretion with no change in serum levels for calcium. Parathyroid hormone caused a rise in serum calcium as well as a rise in calcium excretion, both within 24 hours. The results obtained on these hyperthyroid dogs are in agreement with those obtained on normal dogs (5).

If the thyroid exerts its action on calcium and phosphorus metabolism through stimulation of the secretory activity of the parathyroid gland, one might have expected to find the metabolic responses in the two experiments to be similar. Such is obviously not the case. It is therefore concluded, though not proven, that the thyroid acts in a different manner than through a direct influence on the secretory activity of the parathyroid gland. This conclusion is not vitiated by the fact that the administration of thyroid to thyroparathyroidectomized dogs produced no significant changes in the calcium and phosphorus metabolism. The thyroid hormone may not be able to increase calcium excretion in these animals because of their low serum calcium. It is true that if the effect of thyroid hormone upon calcium and phosphorus metabolism were mediated through the parathyroid gland, this result would be expected. Yet the experimental fact that the feeding of thyroid hormone to the operated dogs produced no significant alterations in calcium and phosphorus metabolism is not, of course, evidence for that hypothesis.

Careful studies on the calcium and phosphorus changes after thyroid administration to thyroparathyroidectomized animals are not numerous. Kunde has made studies on thyroparathyroidectomized dogs, but her data do not include figures for excretion of these substances (7). Neither are studies on the effects of thyroid administration to human patients suffering from hypoparathyroidism to be found in abundance. The most conclusive ones are the very detailed and complete studies of Aub, Albright, and their collaborators (8). These observers gave thyroid to two clinical cases of hypoparathyroidism which were maintained on parathyroid hormone. They found that the thyroid in these cases increased phosphorus excretion, raised the serum calcium, and finally caused an increased urinary excretion of calcium. The latter occurred only after the serum level of calcium had reached about 8 mgm. per cent. They postulate that the calcium excretion in the urine does not rise until the threshold is reached by the level of serum calcium.

It is interesting to note, however, from an examination of their data that, in these same patients, thyroid produced only slight changes in serum calcium and no changes in calcium excretion when parathyroid hormone was not being

simultaneously given. This is in complete agreement with the experimental results of this present study.

The elucidation of these phenomena, and of the mode of action of thyroid here, depends upon an understanding of why thyroid administration to thyroparathyroidectomized dogs does not produce the same marked changes in calcium and phosphorus metabolism as it does in normal animals.

There are several possibilities as to why this occurs. Perhaps the parathyroid hormone must be present in the tissues and fluids of the animal for the demonstration of the characteristic thyroid effect on calcium metabolism. On the other hand, the lower level of the serum calcium in the parathyroidectomized dogs may not allow a rise in calcium excretion when thyroid is given, because the serum concentration is below the calcium threshold of the kidney.

Regardless of the final explanation of why the effects of thyroid and parathyroid administration are dissimilar, it would appear from our results that the effects of the two hormones on calcium metabolism are to be regarded as different both qualitatively and quantitatively.

SUMMARY

1. The administration of thyroid hormone to normal dogs produces a marked increase in calcium excretion, with no change in serum calcium levels. There are no significant changes in phosphorus metabolism.

2. Parathyroid hormone administration to hyperthyroid dogs produces the classical response found to be characteristic of normal dogs.

3. The feeding of thyroid hormone to thyroparathyroidectomized dogs results in only slight increase in calcium excretion in the urine. It does not prevent serum calcium from falling to a subnormal level. There is no manifest effect on phosphorus metabolism.

4. Serum phosphatase remains unchanged throughout such experiments.

5. The implications of these facts are discussed.

The authors wish to thank Professor A. Baird Hastings for his kind assistance in this work and in the preparation of the manuscript. They also wish to thank Dr. S. J. Maddock for thyroparathyroidectomizing the animals.

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TEMPERATURE SENSATION: THE SPATIAL SUMMATION OF HEAT

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It has long been recognized that heat sense differs in its qualities from warmth, but the nature of the physiological process producing the sensation of heat has not been at all clear. The attractive theory of Alrutz, that heat results from a simultaneous stimulation of warmth and cold receptors found support in the work of Dallenbach and his collaborators (1), Cutolo (2), Altson (3) and others. The composite picture presented by these workers was that not only could heat be elicited by simultaneous stimulation of warmth and cold, but also the areas to which these two stimuli were presented could be separated by as much as 10 or 15 cm. and still give a successful synthesis of heat.

Arrayed against acceptance of the Alrutz theory is the work of Heiser, Jenkins and others. Heiser (4) stimulated areas previously established as deficient in cold spots, and could sometimes arouse there sensations of heat. Although this result was not obtained in even a majority of the cases, he considered the evidence good enough to warrant denial of the Alrutz theory. Jenkins (5, 6) worked with a number of untrained subjects and found that attempts to stimulate heat from a synthesis of warmth plus cold were successful only in a scattered minority of cases. In addition, he found (6) if the cold component be replaced by a shock component (electrical shock), that the combination of warmth plus shock was more effective in evoking reports of heat than warmth plus cold.

The present paper offers data on the spatial summation of heat which supports the conclusion that heat sensation is mediated by a receptor type unique to it. It makes untenable the Alrutz theory for heat sensation, although it in no way maintains that heat cannot be elicited by any of the synthetic methods mentioned above.

METHOD. The method was adapted from the radiation technique of Hardy and Oppel (7) and has been described elsewhere (8). Briefly it consisted in focussing the light from a 2,000 watt tungsten lamp onto the blackened forehead of the subject. Stimulus-duration of three seconds was controlled by a manually operated shutter in the light beam. Stimulus intensity was controlled by variable rheostats in the lamp circuit and measured by a radiometer. The area of skin exposed was fixed by suitably selected shields with known circular aperture.

The threshold for heat for three seconds' stimulation was measured for two trained observers (the authors) as a function of area. The intensity of radiation was adjusted until the sensation of heat was evoked just at the end of the three seconds' stimulation for each of the areas studied.

Results for the two observers were highly consistent within the normal spread and each had the same threshold at the various areas. The averages for the data on the two observers are reported in table 1. The spread in threshold intensity from this average was about ±18 per cent for the smallest areas and decreased to about ±7 per cent at the larger areas. Radiation intensities are reported in "units" where one unit is 10^{-5} gm.cal./cm²/sec.

TABLE 1

Thresholds for heat sense on the forehead for different sizes of exposed areas

AREA	THRESHOLD INTENSITY
sq. cm.	*units*
1.0	10,350*
2.0	7,000
3.5	6,050
7.0	4,150
10.0	4,000
15.0	3,550
20.0	2,900
27.3	2,900

* It should be noted that these values were obtained for stimulation lasting three seconds, and they cannot be compared directly with the heat threshold reported in a previous paper (8) in which the stimulus was of two seconds' duration.

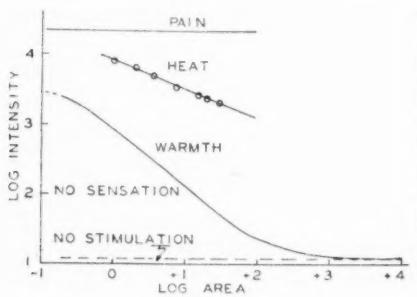

Fig. 1

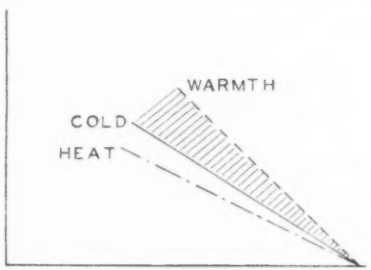

Fig. 2

Fig. 1. Log stimulus intensity vs log area for warmth, heat and pain thresholds, showing the different degree spatial summation for each of these sensations.

Fig. 2. Slopes of the spatial summation curves of heat, cold and warmth plotted from a common point. On the basis of the Alrutz theory, the line for heat should fall somewhere in the shaded region.

The data can be more easily analyzed if they are plotted on a logarithmic scale together with similar data for warmth and pain. Figure 1 shows such a plot. Upon comparing the slope of the heat curve with the corresponding portion of the warmth curve, it is seen that the slopes of the two are markedly different. This means, of course, that heat cannot be the result merely of strong stimulation of warmth receptors, since then the summation should be identical with that of warmth.

The question remains whether or not the cold receptors are concerned in combination with the warmth end-organs. That is, may we consider heat sense, H, to be made up of the sum of the separate components warmth, W, and cold, C? Or, in terms of the threshold sensations, can we say

$$H_0 = K\,(aW_0 + bC_0)?$$

a and b represent the proportions of warmth and cold senses in heat and K is a constant. Should any such relationship exist, a plot of the curve for heat threshold, in terms of $\log A_0$ and $\log I_0$, would show a slope between the curves for a similar plot of warmth and cold. Figure 2 shows the slopes of the log threshold stimulus intensity vs. log area curves for warmth, cold and heat drawn from a common point to allow easy comparison. The Alrutz theory would require that the line representing the slope of the heat curve fall somewhere in the shaded region between warmth and cold. This is not the case. The fact that the line falls outside this region shows that no simple addition of warmth and cold in any proportions will give heat. Indeed, it is hard to see how a positive synthesis of these sensations would give heat, although inhibiting effects and possible complex syntheses are not ruled out. The present evidence is, however, definitely contrary to the Alrutz theory.

Other findings support this view. In a previous paper (8) evidence was presented which showed that a new receptor type was entering into activity as the heat threshold was approached, and that the sensation of heat became apparent when the response of these receptors was intense enough to dominate the sensory experience. The nature of these end-organs was then ambiguous and they were called the "C-receptors." It is now apparent that the "C-receptor" is identical with the heat receptor.

In the same paper (8) it was stated that, whereas for low stimulus intensities the central portion of the forehead seemed to have a symmetrical end-organ distribution, at the high stimulus intensities an asymmetry in end-organ distribution became noticeable. This becomes understandable if one considers that the low intensities excited warmth receptors, while the high intensities excited both warmth and heat receptors, the latter end organs being asymmetrically located over the area under question.

The fact that the skin contains heat receptors which are ready to respond to a thermal stimulus of sufficient intensity deserves emphasis. It has been recognized by Dallenbach and co-workers (9) and Bazett and McGlone (10), for instance, that there exist certain upper limits of stimulus intensity which cannot be surpassed if it be desired to study a single sensation. Their precaution was a wise one. An example will help make clear what is meant: suppose in punctiform mapping, with a view to study warmth sensation, that a stimulus is used which is also adequate for heat. The results will be a mixture of reports on two sensations and not, as is often assumed, on grades of a single sensation. Since these two have different laws of summation, ambiguities will appear when an attempt is made to give an areal interpretation to punctiform mapping tests. Equally ambiguous will be the conclusions upon attempting to compare results

at different levels of stimulus intensity. Thus, the recent results of Jenkins (11) using seriatum mapping with temperatures of 36 and 41°C. are adequately explained on the basis that warmth was stimulated in one case, and warmth and heat in the other. He observed that the mappings at 36°C. were not simple reductions of intensity from those made at 41°C., a result which would be inevitable if heat and warmth were stimulated in the latter case and only warmth in the former.

SUMMARY

1. The threshold for heat as a function of area has been measured for two trained observers using radiation technique. The thresholds for both observers are the same.

2. Heat shows spatial summation, but to a lesser degree than does either warmth or cold.

3. Heat sensation is mediated by its own receptor type and is not a "synthetic sensation" due to warmth and cold.

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DISTRIBUTION OF AVAILABLE WATER IN THE ANIMAL BODY

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This investigation was undertaken to determine the normal distribution of available water in the animal body and the degree to which certain tissues may serve as reservoirs of available water. Isotonic dehydration of two magnitudes and hydration of one magnitude were imposed to determine the stability of the distribution of available water and the degree to which plasma water, available water, and intracellular water volumes are affected by neutral isotonic fluid loss and gain.

METHODS. Full grown, white, male and female rabbits weighing between 2.5 and 3.5 kgm. were used as experimental animals. They had been adequately cared for under similar conditions for several days before determinations were made. Body weight, plasma water and available water volumes, and hematocrit were determined for each animal. Five to seven days later a second determination of these values and the determination of total water and available water content of several tissues were made on each of the same animals under the same conditions. Rabbits were fasted for twelve hours without restriction of water before the determinations were made. Three cubic centimeters of blood were drawn by syringe from the posterior marginal vein of one pinna. Into the same vein 0.15 to 0.20 cc. of a 0.125 per cent solution of Evans blue dye, T-1824, and 0.20 to 0.25 cc. of a 10 per cent solution of sodium thiocyanate per kilogram of body weight were injected from accurately calibrated syringes. The syringes were cleared of dye and thiocyanate by rinsing with blood. Five blood samples of 2.0 to 3.0 cc. each were drawn at approximately twenty minute intervals and four more at half hour intervals from the central artery of the opposite pinna. Approximately twenty units of heparin (Connaught Laboratories, Toronto) were added to each sample of blood. A portion of the blood drawn before injection was centrifuged in a Wintrobe tube, and the red cell and plasma volumes were calculated from the scale readings. The remainder of the first drawn blood and samples drawn after injection were centrifuged. The concentration of dye in the first five or six samples of blood drawn after injection were determined on an Evelyn photoelectric colorimeter using a 620 mμ filter. Corrections were made for the excessive absorption of light whenever there was appreciable hemolysis. One cubic centimeter of plasma from blood drawn before injection of thiocyanate and 1.0 cc. of plasma from each of the samples drawn subsequent to one and one-half hours after injection of thiocyanate were each added to 10.0 cc. of 10 per cent trichloroacetic acid and the

precipitated proteins and dye were removed by filtering. Three cubic centimeters of a 5 per cent acid ferric nitrate solution were added to 7.0 cc. of each filtrate in separate Evelyn photoelectric colorimeter tubes. The concentrations of ferric thiocyanate were determined with a 490 mμ filter. The calculations of plasma water and available water volumes followed the lines suggested by Gregersen and Stewart in 1939.

Available water volumes determined experimentally were lowered 5 per cent to correct for the discrepancy between thiocyanate content of 1.0 cc. of plasma and 1.0 cc. of plasma water. Available water volumes were corrected for the passage of thiocyanate into erythrocytes. Seventy per cent of the total volume of the blood cells, the water content of the erythrocytes, was subtracted from the available water volume. No corrections were made for the concentration of thiocyanate in cells of the salivary glands and gastric mucosa or its slow diffusion into cerebrospinal and synovial fluids. It is difficult to make precise corrections for these variations. The errors introduced by omitting these corrections are small and compensate for one another to a degree. The interstitial water and plasma water volumes are recorded together as available water volume. In the absence of formulae whereby the body surface area of rabbits might be calculated from body weight, plasma water and available water volumes are expressed in relation to the square of the cube-root of body weight in kilograms. The first determinations of body weight, plasma water and available water volumes, and hematocrit and the second determination of these values, made five to seven days later on normal and experimental animals, varied within the experimental range and are not tabulated separately.

Available water volumes of several tissues were calculated on the basis of the amount of thiocyanate in tissues as compared to the amount in plasma water. After the last blood sample was drawn for the determination of plasma water and available water volumes, approximately 1.0 cc. of a 25 per cent solution of sodium thiocyanate per kilogram of body weight was injected into the same vein into which dye and thiocyanate were injected for the determination of plasma water and available water volumes. Two hours later 1.0 to 2.0 cc. of blood were drawn from the central artery of the opposite pinna. Heparin was added and the blood centrifuged. Immediately after drawing the blood sample, the animal was killed by pithing the brain and upper spinal cord directly. Three or more samples of the indicated quantities of the following tissues were transferred to weighing bottles each of which had been previously weighed: 1 to 2 grams of skin (from which the hair had been closely clipped), semitendinosus muscle, rectus abdominis muscle, heart ventricle muscle (the chamber of which had been cleared of blood), and liver, approximately 0.5 gram of spleen, and 3 grams of perinephric, omental, cervical, and inguinal fat. Care was taken to keep the normal amount of blood in the tissues. Fresh weights of all of the tissue samples were determined.

Two-tenths of a cubic centimeter of plasma from the blood sample was added to each of two centrifuge tubes each of which contained approximately 10 cc. of 10 per cent trichloroacetic acid. The precipitated proteins were thrown down

by centrifuging. Each of two samples of each tissue was ground with clean sand and distilled water until the tissue was completely broken up. Macerated tissue and sand were then washed into a centrifuge tube and 1.0 cc. of 50 per cent trichloroacetic acid was added for each 5 cc. of liquid. Precipitated proteins and sand were thrown down by centrifuging. Each supernatant fluid was transferred to a 25 cc. volumetric flask. Each precipitate was broken up and washed with approximately 5 cc. of 10 per cent trichloroacetic acid. The washing was repeated at least twice and each supernatant fluid was added to the corresponding flask. Each fluid was made up to 25 cc. and 10 cc. were transferred to each of two Evelyn colorimeter tubes. Three cubic centimeters of distilled water were added to the first tube and the galvanometer was adjusted to 100 with a 490 mμ filter. Three cubic centimeters of acid ferric nitrate solution were added to the second tube and the concentration of ferric thiocyanate was calculated from the galvanometer reading. By such a comparison reading, errors from turbidity and unprecipitated tissue pigments were avoided. Determinations of plasma and tissue thiocyanate content were made in duplicate. The plasma thiocyanate content was raised 5 per cent to indicate the amount of thiocyanate in 1.0 cc. of plasma water. The available water volume per gram of tissue was calculated from the ratio of the amount of thiocyanate in a gram of tissue to the amount in 1.0 cc. of plasma water.

One sample of each tissue was dried to constant weight at 100°C., the dry weight determined, and the total water content calculated. The intracellular water content of tissues was calculated by subtracting the available water volume from the total water content. The method used for the determination of available water volume in tissues in this investigation had a seemingly high degree of accuracy. In experiments wherein known amounts of thiocyanate were added to tissue, 98 per cent or more of the thiocyanate was recovered.

Using tables published by Skelton in 1927 giving the percentage of the total body weight represented by each of several organs, the percentages of the total available water held by skin, muscle, and liver in a 3 kgm. rabbit were calculated. The average of the available water content of the two somatic muscles analysed was used in the calculations on muscles.

Dehydration of two magnitudes and hydration of one magnitude were imposed on the experimental animals. A moderate dehydration was obtained by injecting a solution containing 0.645 gram of NaCl, 0.255 gram of $NaHCO_3$ and 5.5 grams of glucose in each 100 cc. into the peritoneal cavity. A more advanced dehydration was obtained by injecting a solution containing the same amounts of NaCl and $NaHCO_3$ as in the less severe dehydration and 16.5 grams of glucose in each 100 cc. One hundred cubic centimeters of this solution were injected for each relative unit of body surface area. After four hours a volume of fluid was withdrawn from the peritoneal cavity equivalent to that injected and 22 per cent of the normal available water volume in moderate dehydration, and 50 per cent in advanced dehydration. Moderate hydration was imposed by injecting a solution containing 0.645 gram of NaCl and 0.255 gram of $NaHCO_3$ in each 100 cc. Two hundred cubic centimeters of this solution were injected for

each relative unit of body surface area. After six hours one half of the amount injected was withdrawn. In this way 100 cc. of isotonic NaCl-$NaHCO_3$ solution were introduced into the animal per relative unit of body surface area. Two hours after removal of fluid from the peritoneal cavity in dehydration and hydration experiments, determinations of plasma water and available water volumes and hematocrit were made. After the second imposition of the experimental conditions on the same animal, five to seven days later, a similar equilibration period was allowed, following which plasma water and available water volumes, hematocrit, total tissue water, and available water content of tissues were determined. The fluid injected into the peritoneal cavity was made to resemble the plasma in respect to sodium, chloride, and bicarbonate ions in order not to disturb significantly the electrolyte concentration or acid-base balance of body water.

The loss, or gain, of water in each tissue was calculated on the basis of the increased, or decreased, portion of solid material in the tissues. This calculated loss, or gain, could be verified on the basis of increased, or decreased, portion of intracellular water volume. Therefore, it was apparent that the intracellular water volume was still of the same ratio to solid material as in normal animals. The intracellular water volume, therefore, was not affected and the water loss, or gain, was restricted to the available water compartment. Since no determinations of plasma volume within tissues were made, values were not obtained for the volume and loss or gain of the interstitial water. Using Skelton's tables, loss or gain of water by each of several organs of a 3 kgm. animal was calculated.

Results. Data which were obtained on normal animals are summarized in table 1; on dehydrated animals in tables 2 and 3; and on hydrated animals in table 4.

Discussion. The tissue analyses, presented in table 1, indicate that skin and muscles are the principle depots of available water. Combined they contain three-fourths of the available water in a normal rabbit. Although skin contains approximately four times as much available water per unit weight as muscle does, muscle constitutes about four times as much of the body's weight as skin. Therefore, all of the skin and all of the muscle contain approximately equal amounts of available water. Liver, heart ventricle muscle, and spleen contain more available water per unit weight than somatic muscle does, but due to the fact that they comprise smaller portions of the body's weight, they are depots of lesser significance. Fats are insignificant as storage sites of available water. Apparently fat can be stored practically free of water. The epithelial portion of skin probably does not participate to a great extent in retention of available water. Manery, Danielson and Hastings pointed out in 1938 that connective tissue could be assumed to consist of connective tissue proteins suspended in an ultrafiltrate of plasma. Subcutaneous connective tissue constitutes a considerable portion of the mass of skin. It is probable that in the connective tissue portion is to be found the greater part of the available water store in skin. The larger available water compartment in the rectus abdominis as compared to the semitendinosus muscle may be related to a larger connective tissue fraction in the

TABLE 1

Normal animals

	4 ♂	4 ♀	AVERAGE
Plasma water volume cc. (wgt. in kgm.)$^{-\frac{1}{3}}$	82.7	70.6	76.6
Available water volume cc. (wgt. in kgm.)$^{-\frac{1}{3}}$	325	281	303
Hematocrit (per cent plasma)	67.9	64.8	66.3

Tissue analyses on 4♂ + 4♀

	AVAILABLE WATER, CC. PER GM.	INTRACELLULAR WATER, CC. PER GM.	SOLIDS, GM. PER GM.
Skin	0.627	0.105	0.268
Semitendinosus muscle	0.123	0.633	0.244
Rectus abdominis muscle	0.162	0.563	0.275
Liver	0.340	0.401	0.259
Heart ventricle	0.340	0.439	0.221
Spleen	0.410	0.366	0.224
Perinephric fat	0.043	0.018	0.939
Omental fat	0.066	0.018	0.916
Cervical fat	0.090	0.098	0.812
Inguinal fat	0.110	0.084	0.806

Calculated for a 3 kgm. animal

	AVAILABLE WATER IN ORGANS, CC.	PER CENT OF TOTAL AVAILABLE WATER
Skin	258	41.0
Muscle	219	34.8
Liver	53	8.4
Other organs (calc'd)	100	15.8
Total	630	100.0

TABLE 2

22 per cent of available water withdrawn

	AVERAGE OF 2 ♂ + 2 ♀
Plasma water volume cc. (wgt. in kgm.)$^{-\frac{1}{3}}$	73.2
Available water volume cc. (wgt. in kgm.)$^{-\frac{1}{3}}$	238
Hematocrit (per cent plasma)	64.2

Tissue analyses on 2♂ + 2♀

	AVAILABLE WATER, CC. PER GM.	INTRACELLULAR WATER, CC. PER GM.	SOLIDS, GM. PER GM.
Skin	0.574	0.120	0.306
Semitendinosus muscle	0.102	0.648	0.250
Rectus abdominis muscle	0.134	0.582	0.284
Liver	0.283	0.435	0.282
Heart ventricle	0.290	0.472	0.238
Spleen	0.352	0.402	0.246
Perinephric fat	0.040	0.019	0.941
Omental fat	0.058	0.018	0.924
Cervical fat	0.052	0.103	0.845
Inguinal fat	0.063	0.089	0.848

Calculated for a 3 kgm. animal

	CC. LOST PER GM.	PER CENT OF NORMAL AVAILABLE WATER LOST	CC. LOST BY WHOLE ORGAN	PER CENT CONTRIBUTED OF TOTAL LOSS
Skin	0.125	20	51.5	38.2
Semitendinosus muscle	0.021	17	41.5	30.7
Rectus abdominis muscle	0.032	20		
Liver	0.079	23	12.5	9.3
Heart ventricle	0.070	21		
Spleen	0.090	22		
Perinephric fat	0.003	7		
Omental fat	0.008	12		
Cervical fat	0.040	45		
Inguinal fat	0.050	45		
Other organs (calc'd)			29.5	21.8
Total			135	100.0

TABLE 3

50 per cent of available water withdrawn

	AVERAGE OF 2 ♂ + 2 ♀
Plasma water volume cc. / (wgt. in kgm.)$^{-\frac{2}{3}}$	71.4
Available water volume cc. / (wgt. in kgm.)$^{-\frac{2}{3}}$	153
Hematocrit (per cent plasma)	61.8

Tissue analyses on 2♂ + 2♀

	AVAILABLE WATER, CC. PER GM.	INTRACELLULAR WATER, CC. PER GM.	SOLIDS, GM. PER GM.
Skin	0.378	0.175	0.447
Semitendinosus muscle	0.091	0.656	0.253
Rectus abdominis muscle	0.118	0.592	0.290
Liver	0.267	0.445	0.288
Heart ventricle	0.294	0.470	0.236
Spleen	0.345	0.407	0.248
Perinephric fat	0.042	0.020	0.938
Omental fat	0.062	0.020	0.918
Cervical fat	0.051	0.101	0.848
Inguinal fat	0.067	0.091	0.842

Calculated for a 3 kgm. animal

	CC. LOST PER GM.	PER CENT OF NORMAL AVAILABLE WATER LOST	CC. LOST BY WHOLE ORGAN	PER CENT CONTRIBUTED TO TOTAL LOSS
Skin	0.400	64	165.0	53.0
Semitendinosus muscle	0.035	28	65.8	21.0
Rectus abdominis muscle	0.050	31		
Liver	0.100	29	15.6	5.0
Heart ventricle	0.068	20		
Spleen	0.100	24		
Perinephric fat	0.002	5		
Omental fat	0.005	8		
Cervical fat	0.040	45		
Inguinal fat	0.045	41		
Other organs (calc'd)			65.6	21.0
Total			312.0	100.0

TABLE 4

Available water increased by 34 per cent

	AVERAGE OF 2 ♂ + 2 ♀
Plasma water volume cc. / (wgt. in kgm.)$^{-\frac{2}{3}}$	78.1
Available water volume cc. / (wgt. in kgm.)$^{-\frac{2}{3}}$	407
Hematocrit (per cent plasma)	69.4

Tissue analyses on 2♂ + 2♀

	AVAILABLE WATER, CC. PER GM.	INTRACELLULAR WATER, CC. PER GM.	SOLIDS, GM. PER GM.
Skin	0.695	0.085	0.220
Semitendinosus muscle	0.148	0.615	0.237
Rectus abdominis muscle	0.202	0.535	0.263
Liver	0.400	0.364	0.236
Heart ventricle	0.382	0.411	0.207
Spleen	0.483	0.321	0.196
Perinephric fat	0.050	0.020	0.930
Omental fat	0.074	0.020	0.906
Cervical fat	0.142	0.093	0.765
Inguinal fat	0.176	0.078	0.746

Calculated for a 3 kgm. animal

	CC. GAINED PER GM.	PER CENT OF NORMAL AVAILABLE WATER GAINED	CC. GAINED BY WHOLE ORGAN	PER CENT OF TOTAL GAIN ACCEPTED
Skin	0.225	36	92.7	43.0
Semitendinosus muscle	0.030	24	47.0	21.8
Rectus abdominis muscle	0.050	31		
Liver	0.100	29	15.6	7.2
Heart ventricle	0.070	21		
Spleen	0.140	34		
Perinephric fat	0.007	16		
Omental fat	0.010	15		
Cervical fat	0.060	67		
Inguinal fat	0.080	73		
Other organs (calc'd)			60.7	28.0
Total			216.0	100.0

former. In 1936 Harrison, Darrow and Yannet published estimates of the extracellular fluid content of skin, muscle, and liver. Their method was based on the assumption that chloride ions are limited in their distribution to the extracellular compartment of tissues. The results of the present investigation are comparable to those of these investigators.

The marked constancy of plasma volume even in advanced dehydration, tables 2 to 4, confirms the suggestion made by Underhill and Fisk in 1930 that when there is enough water available to more than offset the concentration of the blood that has developed from the dehydration, it is unlikely that the organism would permit the blood to remain a deficient organ unless the release of the remaining water involved difficulties equally or more serious than those of reduced blood volume. In a state of dehydration amounting to 22 per cent reduction in the available water volume, table 2, the tissues examined, except fats, lost water roughly proportional to their available water content. In the more advanced state of dehydration, table 3, there was no such proportionate loss. When water loss was more severe skin lost a greater fraction of its available water than did other tissues and contributed more than half of the total water loss. The structural stability of organs such as somatic muscles, liver, heart ventricle muscle, and spleen may prevent the removal of more than a portion of their available water. In 1935 Hamilton and Schwartz published results of dehydration studies on force fed, water deprived dogs. The results on total tissue water loss in muscles, skin, and liver in the present investigation are comparable to those of these authors. The animals subjected to the more severe degree of dehydration showed the typical signs of water-loss. They were listless, the mucous membranes of the mouth were dry, and the skin over the body was loose. On opening the peritoneal cavity the surfaces of the viscera were found to be abnormally dry.

An explanation of the constancy of plasma volume in the hydration experiments wherein the available water volume was increased by one-third, table 4, may be found in the two factors involved in the passage of added crystalloid solutions from the vascular compartment into the extravascular compartment: increased capillary blood pressure and decreased effective colloidal osmotic pressure of plasma proteins. In experiments wherein the available water volume was increased by one-third, table 4, the skin accepted an addition of water equal to 36 per cent of its normal available water content. All other tissues investigated, except subcutaneous fat, accepted less in proportion to their normal available water volume. The other tissues were apparently limited by the pressure which resists distention. This pressure was called "tissue tension" by Krogh in 1929. It obviously varies with the anatomical structure and position of the tissue. Such a tension may be a factor in determining the distribution of excessive water in the animal body.

There was no urination by the animals during the experiments and the urinary bladder was found to be only moderately filled in all animals when the peritoneal cavity was opened for tissue sampling. The lack of appreciable urine formation by the animals during these experiments simplifies the interpretation of results

somewhat. The only gain in water was metabolic water and the only loss was by evaporation from the respiratory tract, both of which were assumed to be negligible.

The shift of water across the cell membrane due to disturbances of the osmotic equilibria and physiological neutrality has been a factor in the investigations of dehydration and hydration reported in the literature. In the earlier investigations administration of NaCl caused an increased concentration of extracellular electrolytes resulting in an increase in the volume of extracellular fluid at the expense of intracellular fluid. Administration of glucose resulted in dilution of the extracellular electrolytes which caused a shift of water into the intracellular compartment. In 1937 Eichelberger and Hastings demonstrated that when the acid-base balance is disturbed with a resulting acidosis, there is an increase in the volume of extracellular fluid of muscle. When there is a resulting alkalosis, there is an increase in both extracellular and intracellular fluid volumes.

In 1939 Manery and Hastings suggested that it may not be possible to divide tissues into those which do take up thiocyanate ions and those which do not, but that several tissues may contain cells which take up varying small amounts. The amount of thiocyanate accepted by the intracellular phase of the tissues of rabbits studied in this investigation seemed to be insignificant. The direct proportionality between the percentage of available water lost or gained and the percentage of the total water lost or gained by the tissues indicates no discrepancy due to the passage of thiocyanate into the cells.

Plasma water volume changed only slightly in all of the experiments. Since, however, the available water volume was altered during dehydration and hydration, these changes can be accounted for only by assuming a decrease and an increase of interstitial water volume. Such changes in interstitial water volume are indicated by the data in tables 1 to 4. Actual values may be obtained by subtracting plasma water volume from the available water volume. The mechanisms of circulation of blood make maintenance of normal volume of vascular fluid more imperative than maintenance of interstitial fluid volume. The volume of the interstitial compartment exhibits a wide range of adjustability in defense of plasma volume. Available water may be considered as a single fluid lying in two compartments equipped with mechanisms, effective osmotic pressure of plasma proteins and hydrostatic pressure of blood in the capillaries, for producing movement between the interstitial and vascular compartments. It is apparent from this investigation that tissues differ markedly in their content of available water and that some tissues participate more than do others in maintaining a normal and constant plasma volume.

CONCLUSIONS

Skin and muscle combined contained three fourths of the available water in normal rabbits. Plasma water volume was strikingly protected by the interstitial portion of available water under conditions of moderate and advanced dehydration and hydration. Skin was found to be the most flexible of the available water reservoirs. In moderate dehydration skin, muscle, liver, heart

ventricle muscle, and spleen lost water in proportion to their available water content. In advanced dehydration skin lost a greater fraction of its available water than did any other tissue. In hydration skin accepted almost half of the added water. Intracellular water volume was not affected by loss or gain of neutral isotonic fluid by the available water compartment.

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THE CENTRAL PATHWAY FOR THE JAW-JERK[1]

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Reflex jaw opening to noxious stimulation and reflex jaw closing accompanying swallowing were described by Sherrington (1917) as occurring in the decerebrate cat. In addition to these movements, the jaw of a decerebrate or lightly anesthetized animal exhibits a jerk reflex to striking the lower jaw. The evidence indicates that the jaw-jerk is a true stretch reflex, and it must therefore be initiated by proprioceptive stimuli. Many workers, from Johnston in 1909 to Corbin in 1940, have suggested on morphological grounds that it is the mesencephalic root and nucleus of the trigeminal complex that receive the proprioceptive impulses from the muscles of mastication. This was verified by Corbin and Harrison (1940), who set up proprioceptive impulses by stretch of the masticator muscles and traced those impulses into the mesencephalic root with the aid of a cathode ray oscillograph.

Since the findings listed above do not support the work of Bremer (1923) and Rioch and Lambert (1934), we have attempted to obtain more accurate information concerning the pathway followed by the jaw-jerk reflex.

METHODS. The experiments were carried out on 36 adult cats. In 17 animals, small electrolytic lesions were placed in the mesencephalic root 2 to 23 days before the final observations were made. The root was located using a Horsley-Clarke instrument and recording action potentials with a cathode ray oscillograph. It was then possible to place a lesion in the root between the motor nucleus and the caudal part of the mesencephalic nucleus (fig. 1). With a 3 ma. direct current for 30 seconds the lesion was placed on the right side, the left root being retained as a control. At the time for final observation, each animal was decerebrated (under ether anesthesia) just rostral to the superior colliculus by the trephine method. Time was allowed for the development of decerebrate rigidity, and the jaw reflexes then were studied. Needle electrodes were placed in the masseter muscle for recording. The teeth and gums were stimulated faradically, and with blunt pressure or light sharp taps. The oral mucosa was stimulated faradically. A procedure similar to the one outlined above was also carried out in 6 acute experiments. In other experiments, the mesencephalic, chief sensory and spinal nuclei and various peripheral

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branches of the fifth cranial nerve were stimulated with single and multiple pulses from a thyratron stimulator. In cases of central stimulation, orientation was effected by the Horsley-Clarke instrument. In 3 of the experiments

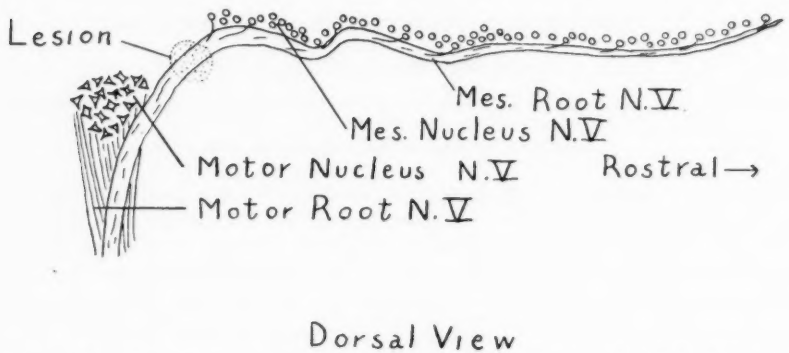

Fig. 1. Dorsal reconstruction of the mesencephalic and motor nuclei of the fifth cranial nerve and their roots.

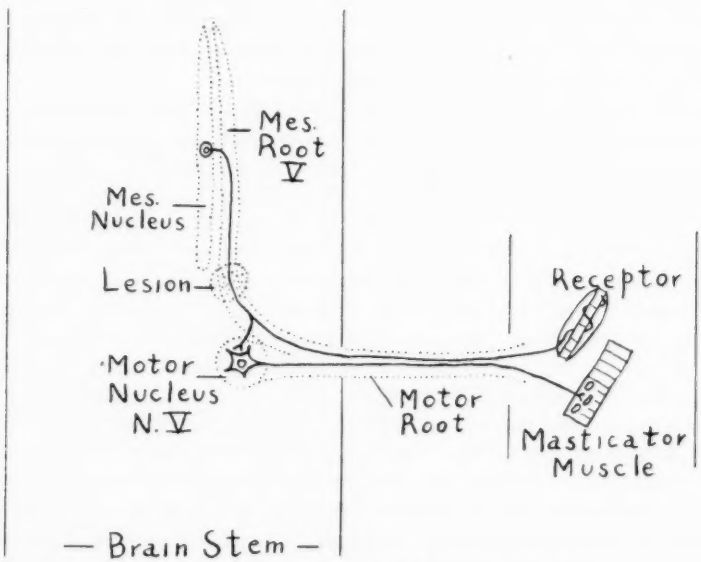

Fig. 2. Diagram of proprioceptive reflex arc of masticator muscles.

measurements were made of the reflex time following stimulation of the mesencephalic root. The brain of each animal was removed and the proper portions of the brain stem placed in a solution of alcohol, dioxan and toluidine blue for fixation and staining. Frozen sections were cut at 50 microns and studied microscopically for location of electrode positions and sites of lesions.

OBSERVATIONS. It was found that a discrete lesion involving the caudal portion of the mesencephalic root of the fifth cranial nerve would abolish the jaw-jerk on the side of the lesion (figs. 3, 4 and 5) but had no effect on the other

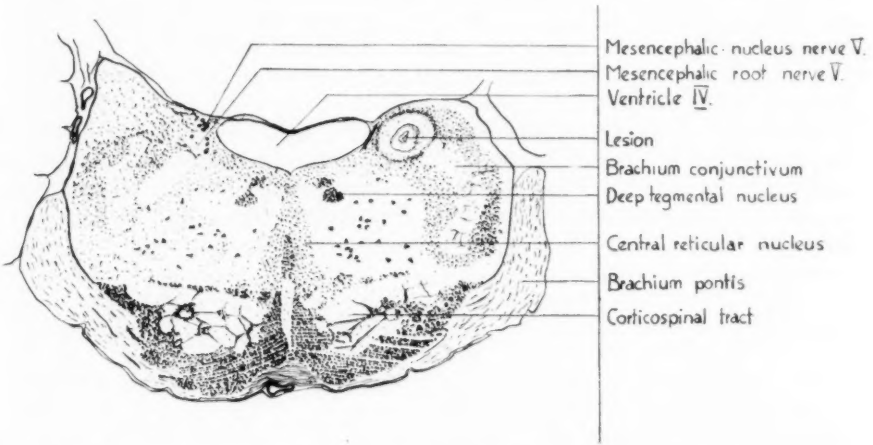

Fig. 3. Section of brain stem of cat 10 through rostral part of pons showing complete destruction of mesencephalic root and nucleus in that region. The jaw-jerk was abolished in this animal. The plane of section for this figure and for figures 4 and 5 is at a 35° angle to the vertical plane of the Horsley-Clarke coördinate system.

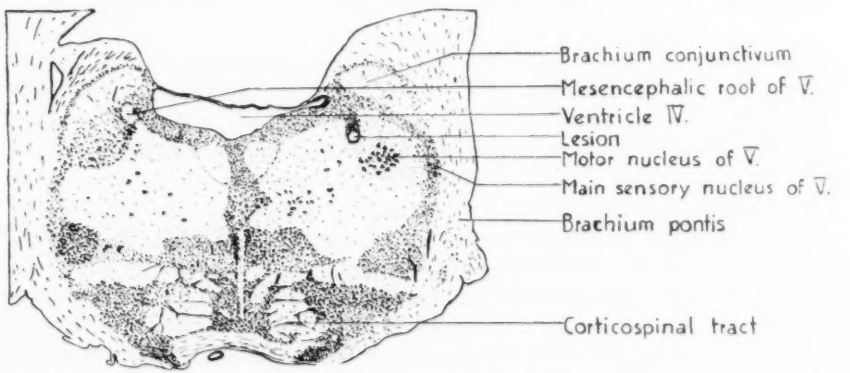

Fig. 4. Section of brain stem of cat 10 somewhat caudal to figure 3, showing caudal extent of the lesion and freedom of the motor nucleus from damage.

jaw reflexes such as jaw closing with reflex swallowing or jaw opening to noxious stimulation. The abolition of the jaw-jerk was unilateral and the reflex was normal on the side of the intact mesencephalic root. Due to the accurate localization afforded by the Horsley-Clarke instrument, there was no damage to the motor nucleus, chief sensory nucleus or spinal nucleus of the fifth nerve.

This was verified by histological study of the cells of the nuclei and by the fact that jaw reflexes other than the stretch reflex were intact bilaterally. In cases where the lesion was placed more rostrally, so that the most caudal parts of the mesencephalic nucleus were spared, the jaw-jerk on the side of the lesion was present but was considerably less in magnitude than on the intact side. In a few experiments the lesion was placed caudally but spared either the more medial or the more lateral fibers of the mesencephalic root. In such cases the jaw-jerk was still present but attenuated.

In decerebrated or lightly anesthetized animals, jaw movements other than the jaw-jerk can be elicited. Light taps on the teeth, especially on the canines, elicit jaw movements. In any one animal the type of movement was rather constant, but responses from several animals were about equally divided between jaw opening and jaw closing. In sensitive preparations tapping the zygoma or other bony prominence elicited jaw closure, but this response is likely

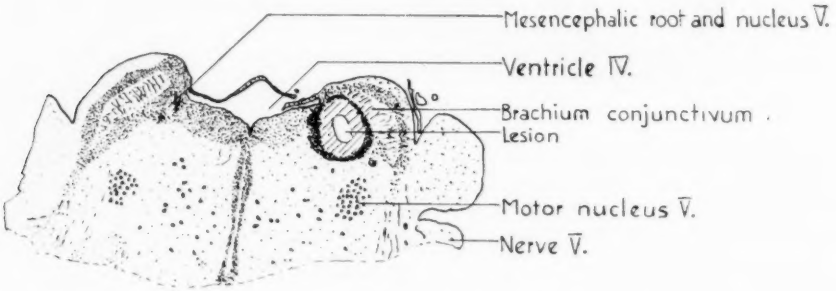

Fig. 5. Section of brain stem of cat 16 showing complete destruction of caudal part of mesencephalic root and the intact motor nucleus. The jaw-jerk was abolished in this animal.

of the type described by Sherrington (1898) as a "jar" reflex. Faradic stimulation of the gums, teeth, palate, oral mucosa and sometimes the surface of the tongue uniformly provoked jaw opening, and the response was unaffected by lesions in the mesencephalic root. Blunt pressure on the teeth or anterior part of the hard palate elicited jaw opening. Stimulation of the cut central end of the superior alveolar nerves produced only jaw opening, even at very low intensities of stimulation. Stimulation of the spinal root and nucleus and chief sensory nucleus consistently occasioned jaw opening, usually by active contraction of the jaw opening muscles but sometimes simply by inhibition of the masticator muscles. Stimulation of the various parts of the mesencephalic root at frequencies of one shock in 2 seconds to 1000 per second yielded only jaw closure. At low frequencies each stimulus evoked a quick sharp closure with immediate relaxation and at higher frequencies there was tetanic fusion, but reversal was not seen. The response was strictly unilateral.

When the stimulating electrode was in the fifth cranial motor nucleus, the time from shock artifact to the beginning of the muscle action potential was

1.7 to 2.3 msec. with most of the observations 2 msec. or less. When the stimulating electrode was in or near the mesencephalic root, the time of response varied with stimulus strength, position of the electrode and condition of the animal. The delay (stimulus artifact to muscle potential) ranged from 2.6 msec. to 6 msec. in the various determinations. Upon moving the stimulating needle from the mesencephalic root to the motor nucleus the difference in time for the appearance of the muscle action potential was in one experiment 0.9 msec. (2.6 in root, 1.7 in motor nucleus), in another experiment, 1 msec. (3 in root, 2 in motor nucleus) but in other determinations the time was as long as 3.8 msec. (5.6 in root, 1.8 in motor nucleus). In the experiment in which the time for appearance of the muscle action potential was 5.6 msec., two stimuli 3.7 msec. apart elicited a response 2.6 msec. after the second stimulus, a decrease of 3 msec. in response time. Sometimes a single shock, even when quite strong, would not evoke a response but two successive stimuli of less magnitude would elicit one. It was not often, however, that a single stimulus applied close to the mesencephalic root failed to bring about jaw closure.

Discussion. The caudal portion of the mesencephalic root may be interrupted close to the motor nucleus without damaging any other component of the fifth complex (fig. 1). As the nerve fibers leave the brain stem, the motor fibers are located ventromedially, the fibers going to the chief sensory nucleus are dorsolateral, and the mesencephalic root fibers are intermediate in position. It is not until a few millimeters from the brain stem that the mesencephalic root fibers are completely mixed with the motor fibers. It follows, therefore, that proof of intactness of motor fibers following operations on the trigeminal nerve near the brain stem does not signify unimpairment of the mesencephalic root fibers.

Two papers have presented evidence that the mesencephalic root is not involved in the jaw-jerk. Bremer (1923) described failure of the jaw-jerk to disappear when the brain stem was transected just caudal to the inferior colliculus; Rioch and Lambert (1934) described abolition of the jaw-jerk by section of the sensory root near the brain stem. Two explanations can be given for Bremer's results. One is that by a transection caudal to the inferior colliculus, the collaterals from the mesencephalic root to the motor nucleus, described by Cajal (1909), were not interrupted, and the collaterals may well have carried on reflex activity for some hours. However, it may be that the level of transection failed to interrupt the connections between the caudal mesencephalic nucleus cells and the motor nucleus. It was found in the experiments reported here that the most caudal portion of the mesencephalic nucleus was sufficient to maintain reflex jaw closing, though the response was decreased in comparison with the normal side.

Rioch and Lambert described a loss of the jerk reflex upon section of the sensory root and stated that the motor root was intact because it still responded to stimulation. It is unlikely that the sensory root could have been completely severed without some damage to the mesencephalic root fibers lying between it and the motor root near the brain stem. Such damage would be undetected

by subsequent stimulation of the motor fibers. Furthermore, the fact that direct electrical stimulation of the motor root still evoked a response does not necessarily indicate that reflex activity over the same nerves would be present.

In the present experiments the tip of the needle was placed at the desired part of the mesencephalic root with little other operative procedure than trephining. A direct current permitted a transection of the root to be produced with slight or no damage to surrounding structures. The chronic experiments were designed to allow degeneration of collaterals to the motor nucleus and thus obviate one of the objections to earlier work.

Data from other types of experiments, and those presented here indicate that the mesencephalic root of the fifth cranial nerve forms the afferent pathway for the jaw-jerk. This is supported by the fact that stimulation of the mesencephalic root with single shocks elicited a quick brief closure similar to the quick closure of the jaw-jerk whereas stimulation of no other sensory trigeminal nucleus gave such a response. On the other hand, stimulation of the spinal or chief sensory nuclei evoked only jaw opening. This latter observation, in addition to the others, makes untenable the statement of Rioch and Lambert that "The afferent path for the jaw-jerk in the decerebrate cat is through the Gasserian ganglion and the sensory root."

Pfaffmann (1939) recorded slowly adapting impulses traveling 24 to 60 meters per second in the superior alveolar nerve and indicated that the impulses arose in the periodontal membrane of the upper teeth. We have recorded similar impulses in the inferior alveolar nerve upon applying pressure to the lower anterior teeth of the same side. Corbin and Harrison (1940) recorded impulses in the mesencephalic nucleus due to blunt pressure over the homolateral upper teeth and palate; these impulses were presumably similar to ones recorded peripherally by Pfaffmann and were traveling over the fibers described by Corbin (1940) as passing from the region of the teeth to the mesencephalic root. Since blunt pressure over the teeth elicits jaw opening and since blunt pressure provokes increased activity in the mesencephalic root, an inhibitory function has been attributed to those fibers from the mesencephalic root to the teeth (Corbin, 1940; Corbin and Harrison, 1940). The failure to induce jaw opening by stimulation of the mesencephalic root in the experiments reported here may be ascribed to the predominance of jaw closing afferents in the root from the masticator muscles and to the more limited distribution in the root of fibers from the teeth (Corbin, 1940). Subjectively, the force of the bite may be inhibited by a sensation of unusual pressure on the teeth or by painful sensations from oral structures. The rhythmic inhibition by pressure developed during chewing (Sherrington, 1917) is a somewhat different mechanism from the complete cessation of chewing due to painful sensation from oral structures.

In the description by Sherrington (1898) of reflexes elicited by percussion, it was pointed out that a tap may set up afferent impulses at a distance from the point of impact simply by a transmitted jarring of distant bony parts. This must be the explanation of the presence of the jaw-jerk upon tapping the zygoma or, as was noted by Rioch and Lambert, by tapping the skull.

The increase in time for response of the masticator muscles upon shifting the stimulating electrode from motor nucleus to mesencephalic root was approximately 1 msec. but was 3.8 msec. in one experiment. The shorter time indicates one synapse between mesencephalic root and motor root but the longer times suggest the presence of interneurones (Renshaw, 1940). Since many responses allowed time for only one synapse, it is evident that the collaterals from the mesencephalic root to the motor nucleus, which were described by Cajal (1909), May and Horsley (1910), Weinberg (1928) and others, can fire the motoneurones directly without the interpolation of interneurones (fig. 2). Johnston (1909) pointed out that the anatomical arrangement of the root and its collaterals to the motor nucleus would permit such a direct reflex. It is this pathway that may remain functional for a short time after lesions are placed rostral to the origin of the collaterals.

SUMMARY

Lesions in the mesencephalic root of the fifth cranial nerve which transect the root just rostral to the motor nucleus abolish the jaw-jerk on the side of the lesion. Lesions which partially destroy the caudal portion of the root depress the jaw-jerk.

Electrical stimuli applied to the mesencephalic root cause a contraction of the masticator muscles on the same side. A single stimulus produces a single contraction of the homolateral muscles; the motor impulses appear after a synaptic delay of about one millisecond.

Electrical stimuli applied to the chief sensory nucleus or spinal root and nucleus of the fifth cranial nerve usually elicit active jaw opening.

The authors wish to express their appreciation to Mr. Kurt Elias who very kindly helped with the illustrations for this paper.

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AN INVESTIGATION OF INHIBITION BY DIRECT STIMULATION OF THE TURTLE'S HEART[1]

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Inhibition of the heart of Limulus by interrupted currents of sufficient strength was described by Carlson (1). In this species, rhythmicity and automaticity are properties of the ganglionated cord and inhibition was produced by direct faradization of the ganglion.

Erlanger (2) demonstrated a similar inhibition by direct stimulation of strips of vertebrate heart and developed a theory of inhibition from this finding.

Relationships of frequency and intensity of stimuli to excitation and inhibition of the cardiac ganglion of Limulus were investigated by Garrey and Knowlton (3). The relative simplicity of the arrangement in the heart of Limulus made it possible to localize the effects in the cells of the ganglion and rule out effects on muscle and neuro-muscular junctions. The possibility that a humoral mechanism was involved was recognized but seemed difficult to harmonize with the shift of reversal point with change of temperature.

It seemed of interest to investigate the matter further using the vertebrate heart preparation since the intimate relation of a humoral mechanism to vagus inhibition in this tissue has been so well established by the work of Loewi and his co-workers (4). As the results of the present investigation differed, depending on whether auricular or ventricular tissue was employed, they are described separately.

EXPERIMENTAL. *Auricular tissue.* The auricles were removed from the turtle heart and their contractions recorded on a kymograph in the usual manner. Sinus tissue was usually included and served as pacemaker of the preparation. Suspension arrangement was such that the tissue could be immersed in test solutions as desired. Break shocks of selected frequency, supplied by a variable speed motor-driven stimulator, were applied by means of non-polarizable wick electrodes. In some experiments, condenser discharges from a Campbell (5) stimulator were employed with identical results. Frequencies up to about 50 per second were employed. Effects of higher frequencies were not investigated. Beginning with slower rates, results of increasing the frequency and of variation in intensity were recorded.

The initial effects observed were found to vary with the strength of the break shocks employed. Stimuli which were just subliminal if single, when applied

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repetitively caused a diminished amplitude without any change in rate and this diminished amplitude may progress to complete inhibition without preliminary excitation as in figure 1, A and B. Under these conditions, inhibition appears to be the primary and sole effect of the applied currents. With break shocks of greater intensity, the initial effects were those commonly observed

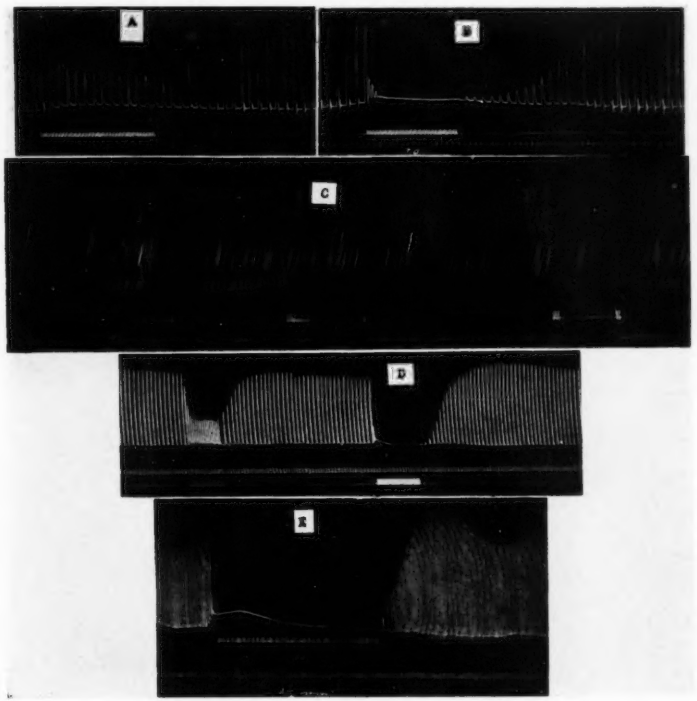

Fig. 1. Record from the double auricle preparation. Upper signal indicates time and rate of stimulation. Lower signal indicates time in seconds.

A. Inhibition of amplitude without change of rate. Stimulation frequency = 6 per sec. B. Same preparation. Complete inhibition with increase of intensity. C. Four successive increases in frequency of stimulation. Intensity greater than in A. Frequencies = 1 per 2 sec.; 1 per sec.; 2 per sec.; 3 per sec. Kymograph speed was quickened momentarily to register frequency of stimulation. D. Effects of stimulation rates of 1 per sec. and 6 per sec. Intensity as in C. E. Well marked supernormal phase of recovery after 2½ min. inhibition.

in cardiac tissues. At stimulator frequencies lower than that at which the tissue was contracting, extra systoles appeared. At increased frequency, the auricles followed the new rhythm. Next, blocking appeared and every second or third shock was effective. With still greater frequency, a few irregular contractions were followed by complete inhibition. These changes are seen in figure 1, C, D, E.

Within a certain frequency-intensity range, complete inhibition followed either increased frequency without intensity change or increased intensity with unchanged frequency.

It seems possible to continue this inhibition for an indefinite period. This point was not investigated specifically but inhibition was maintained for periods of five minutes to be followed by the usual recovery on cessation of the stimulation. Recovery from inhibition was always gradual, the effect disappearing

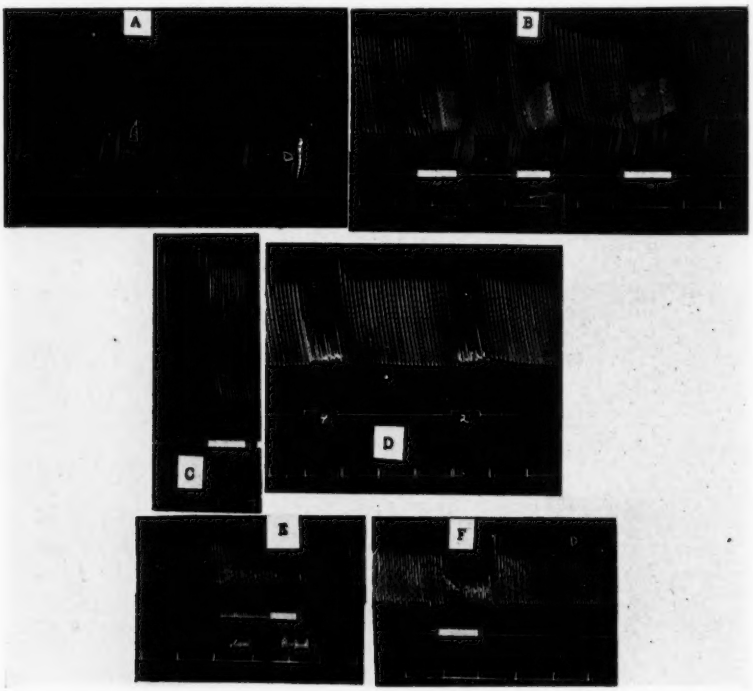

Fig. 2. A and B. Atropinized double auricle preparation. Stimulation at different frequencies and intensities. Upper signal indicates stimulation. Lower signal = time. A = sec. B = ½ min. C, D and E. Records from ventricle stimulated at similar frequencies and intensities. Time = ½ min.

slowly to be followed in most instances by a supernormal phase of increased amplitude of contraction. Recovery and supernormal phases are illustrated in figure 1, C, D and E.

If the above described inhibition produced by direct repetitive stimulation involves a humoral mechanism similar to that of the vagus, with liberation of acetylcholine, it was reasoned that atropine should diminish or abolish the inhibition and eserine should potentiate the effect. To test these possibilities the auricular preparation was thoroughly atropinized by immersion in an atropine sulphate-Ringer solution or by injection of atropine sulphate into the

turtle a short time before removal of the auricle. Following this procedure, repetitive stimulation no longer caused inhibition. The auricles responded to lower rates and intensities by acceleration. With faster rhythms there was partial blocking often with irregular contractions as different fibers recovered at different rates. Strong intensities usually precipitated a state of fibrillation. On cessation of the stimulation, the original intensity and rhythm were resumed after a brief compensatory pause, figure 2, A, B and C.

In contrast to atropine, eserine was found to reinforce inhibition. When repetitive stimuli were applied after immersion in an eserine sulphate-Ringer

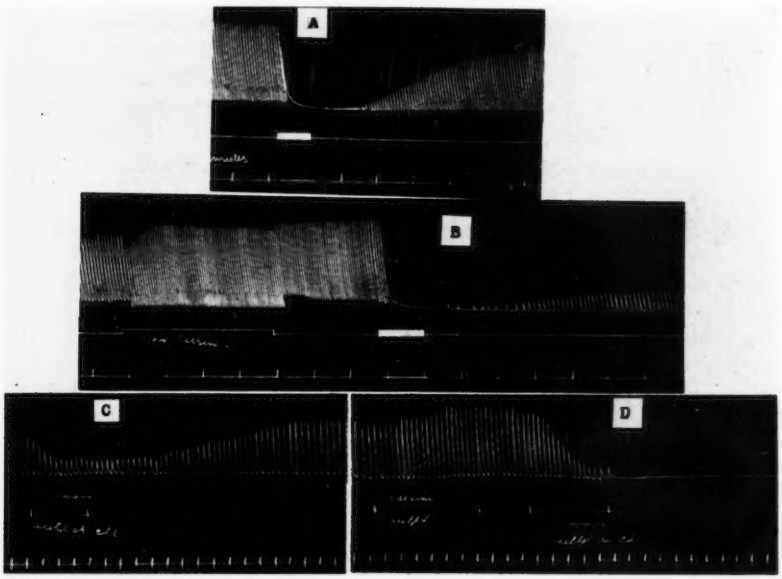

Fig. 3. Double auricle preparation. A = inhibition before eserine. B = effect of eserine on inhibition and recovery. Time = ½ min. C and D. Comparative effect of 1–1,000,000 acetylcholine chloride before and after eserine. Time = 5 sec.

solution, lower intensities and frequencies were effective and inhibition was prolonged. After thorough eserinization with a 1-1000 eserine sulphate solution, recovery from subsequent inhibition was never complete. After a lengthened period of inactivity, feeble contractions were resumed but never reached former amplitude or frequency, figure 3, A and B. After immersion in 1-500 eserine sulphate solution, there was usually no recovery from subsequent inhibition. These effects of eserine could be prevented or abolished by atropinization. In addition, it was possible to show that the inhibitory effects under investigation could be quite closely paralleled by brief immersion in solutions of acetylcholine of appropriate strength, figure 3, C and D.

Ventricular tissue. The series of experiments with auricular preparations were

repeated using the turtle's ventricle. In no case could inhibitory effects be produced by direct stimulation applied either to the whole ventricle or to strips of ventricular tissue.

Like the atropinized auricle, the ventricle followed lower frequencies and intensities and passed into a state of fibrillation with higher intensities. Eserine had no potentiating influence to cause subsequent inhibition. Further, direct application of, or immersion in acetylcholine in any concentration which may be termed physiological, was without effect. This is complete confirmation of the finding of Garrey and Chastain (6) that acetylcholine and related substances have no inhibitory action on the turtle's ventricle and supports the view that the turtle ventricle possesses no mechanism for its direct inhibition. Effects of ventricular stimulation are shown in figure 2, D, E and F.

Discussion. Reversal of the character of the response, i.e., change from excitation to inhibition, with change in the character of the stimulation or change in the physiological state, has been observed in many tissues. Probably entirely different mechanisms are involved in different cases. Selective excitability of different nerve fibers may explain some reversals as in peripheral vasomotor changes. Such a reversal from vasoconstriction to vasodilatation was described by Ostroumoff (7) and later confirmed by Bowditch and Warren (8). A Wedensky effect (9) may be the basis of some reversals. Humoral mechanisms may be involved in other cases. Pharmacological evidence presented above including abolition of inhibition by atropine, its reinforcement and prolongation by eserine and its resemblance to effects of direct application of acetylcholine strongly support the conclusion that the inhibition produced by repetitive stimulation is humoral in character. Acetylcholine is released in a manner similar to that associated with vagus stimulation and most likely from the same source. Inhibition from a single induction shock or condenser discharge was never observed. If of sufficient strength to cause any effect, it was always excitation.

When applied repetitively, the electrical stimuli may cause the release of acetylcholine in progressively larger amounts and a concentration sufficient to inhibit is rapidly reached. Irritability is so depressed that normal rhythm is suppressed and direct excitation becomes ineffective. As the acetylcholine is destroyed the tissue slowly recovers. This recovery is delayed or prevented by eserine. By proper intensity-frequency relationship, it is possible to get inhibition without the usual preliminary excitation. The above inhibitory effects cannot be produced in turtle ventricular tissue which, as has been noted, is not sensitive to acetylcholine and is without vagus endings.

In a number of respects the inhibition under investigation resembles that produced by direct stimulation of the ganglionated nerve-cord of the heart of Limulus, yet one is not justified in concluding that the mechanism involved is necessarily the same. In experiments on the neurogenic heart of Limulus previously cited, stimuli were applied directly to the ganglion and the ganglion cells were directly inhibited. The cardiac muscle cells were not directly depressed, indeed their irritability tested by direct stimulation was found to be augmented. Further, according to Carlson, atropine was without effect on

this inhibition. On the other hand, in the turtle's auricles, applied current may act on ganglion cells, sympathetic and vagus nerve terminals, and on muscle. Ultimately the muscle cells are inhibited.

SUMMARY

The change from excitation to inhibition as a result of repetitive application of electrical stimuli to the auricular muscle of the turtle and the effective frequency-intensity relationships have been investigated.

Inhibition is prevented by atropine and reinforced and prolonged by eserine.

The inhibition and subsequent recovery are similar to that following vagus excitation or immersion in acetylcholine solution.

Above facts are presented as evidence that the repetitive stimulation probably affects vagus terminals selectively and causes liberation of acetylcholine in quantity sufficient to play an essential rôle in lowering excitability and abolishing contractility.

The ventricle muscle which is not inhibited by acetylcholine and which probably receives no vagus fibers does not show this type of inhibition.

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THE EFFECT OF THYROXINE ON THE SENSITIVITY OF THE NICTITATING MEMBRANE OF THE CAT

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The investigations of Asher and Flack (1910) led them to conclude that thyroid secretion sensitizes sympathetic nerve endings to adrenaline as applied to circulatory responses. Oswald (1915) used iodothyroglobulin in similar experiments and reached the same conclusion. Following Kendall's isolation of thyroxine, Levy (1916) also found this agent able to produce an increased pressor response to adrenaline. Sawyer and Brown (1935) tested the influence of thyroxine on the denervated heart and showed that it sensitizes that organ to adrenaline. These studies on cardiac muscle and the responsive elements in the vascular system raise the question whether other structures activated by adrenaline may be sensitized to that agent by thyroxine. In the present study the nictitating membrane was used toward obtaining an answer to that question.

METHODS. Cats were used, under nembutal anesthesia (Abbott, 0.7 cc. per kgm.). Records were taken repeatedly from the same animal, usually at 2-day intervals. The head was fixed in a Czermak head holder. The contractions of the nictitating membrane were recorded isotonically on a kymograph. The head holder, the lever and the writing point were left strictly in place throughout a series of tests. Care was taken to set the cat's head in the same position and to attach the lever (by means of a *serrefine*) at the same point on the membrane, and to the same degree, in successive recordings. In some animals, the sensitivity was tested by means of adrenalin (Parke, Davis). Doses of 0.025 to 0.400 mgm., made up fresh in 1.0 cc. of normal saline, were injected into the unexposed saphenous vein. Each injection lasted 10 seconds. In other animals, repetitive shocks were applied to the cervical sympathetic from the secondary of a Harvard induction coil. In addition, the cervical sympathetics of another group of animals were stimulated by short rectangular pulses from a multivibrator at frequencies of 1, 3, 7, 10 and 15 per second.

Control responses of the nictitating membrane to adrenaline or to sympathetic stimulation were recorded on two separate days. Then thyroxine (Thyroxin Fraction, Squibb) was administered in doses of 6, 10, 20, 30 or 40 mgm. Thereafter the responses to adrenaline or nerve stimulation were again recorded at various intervals from 2 days to 4 weeks.

Isometric records were obtained with 5 animals and used to check against the isotonic records. The nictitating membrane was connected by means of a

[1] Fellow of the Rockefeller Foundation.

serrefine and thread to a torsion spring on which was soldered a small mirror. A beam of light was directed to the mirror so that the reflected beam fell on the zero point of a centimeter scale 2 meters away. Contractions of the nictitating membrane following injection of adrenaline or stimulation of the cervical sympathetic were then recorded as deflections of the reflected beam along the scale.

Pituitary thyrotropic hormone (Antuitrin T, Parke, Davis) was injected into normal animals in order to test the ability of natural thyroid secretion to sensitize the nictitating membrane. The doses of Antuitrin T used were 0.1, 0.2, 0.3, 0.6 and 1.0 cc. Here again control responses were recorded before the administration of the hormone. Thyroidectomized animals, subjected to the same procedures, served as controls for any action that Antuitrin T might have independent of its thyrotropic effect.

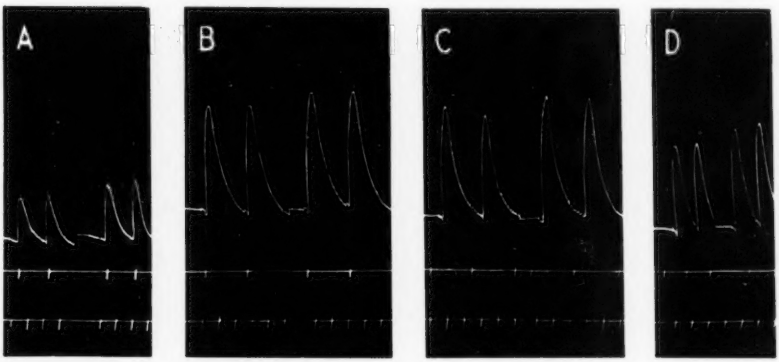

Fig. 1. Increase of response of the nictitating membrane to adrenaline after thyroxine. Records of isotonic contractions produced by intravenous injections, at the upper signals, of the following doses of adrenaline: 12, 12, 16 and 16γ. Time in 1-min. intervals. A, control, before thyroxine. B, 6 days after administration of 20 mgm. thyroxine. C, 10 days after thyroxine. D, 20 days after thyroxine.

Results. A. *Sensitization to adrenaline.* Small doses of thyroxine (up to 6.0 mgm.) had no significant effect on the responses of the nictitating membrane to adrenaline or nerve stimulation.

In 11 animals, doses of 10 to 20 mgm. caused an increased amplitude of contraction of the membrane after adrenaline. With 10 mgm., this increase was evident by the 48th hour, reached its peak on the 3rd day and fell to control levels within 14 days. The percentile increases at the peak ranged from 69 to 110 per cent. Doses of thyroxine of 20 mgm. resulted in an earlier increase of the responses to adrenaline, i.e., by the 20th hour. The peak of the effect, from 100 to 160 per cent increase, occurred on the 6th to 9th day and the subsidence to control levels on the 21st to 25th day.

The effect of a 20 mgm. dose of thyroxine is illustrated in figure 1. These results are typical of the control responses and those 6, 10, and 20 days after thyroxine. All responses were considered as having returned to normal when

within 1 to 2 mm. of the controls. This standard deviation was selected on the basis of 20 measured variations in controls.

That the action of thyroxine in producing an increased sensitivity of the membrane is not dependent on a tonic discharge via the cervical sympathetic was shown as follows. The nictitating membranes were denervated in 3 animals and then tested for the maximum increased sensitivity due to denervation, between the 15th and 17th day thereafter (Hampel, 1935). Administration of thyroxine to these animals produced an increased reaction to all doses of adrenaline beyond the maximal responses due to denervation.

The sensitizing effects following administration of 30 to 40 mgm. of thyroxine to 6 animals were less striking than with 20 mgm. The peak of the effect took

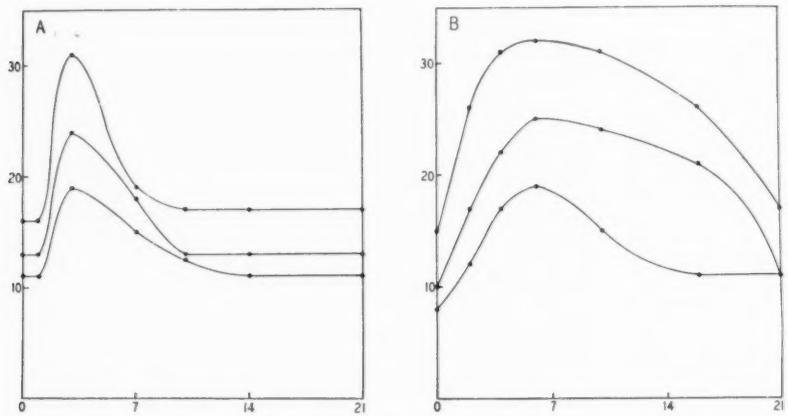

Fig. 2. Time course of the sensitizing action of thyroxine on the response to adrenaline. Ordinates: responses in millimeters. Abscissae: days after administration of thyroxine. A, results following 10 mgm. dose of thyroxine. B, effect of 20 mgm. dose of thyroxine. Upper curve: 40γ of adrenaline. Middle curve: 20γ of adrenaline. Lower curve: 12γ of adrenaline.

place about the 4th day after administration and the increase was from 14 to 56 per cent. The animals to which these large doses were given usually died after 4 to 6 days.

In figure 1, as previously noted, are shown typical increments of the responses to two standard doses of adrenaline (12 and 16γ) after a single administration of thyroxine (20 mgm.). Characteristic effects of 10 and 20 mgm. of thyroxine are plotted graphically in figure 2. Although the absolute increase of response was greater for larger than for smaller doses of adrenaline, the percentile increment in the early stages after the administration of thyroxine was approximately the same for the three doses of adrenaline illustrated.

Figure 3B presents in more detail the degree of sensitization for several doses of adrenaline obtained in an animal 6 days after 20 mgm. of thyroxine had been injected. The lower curve shows the amplitude of the control responses to

different doses of adrenaline before thyroxine was given, the upper curve (dots), the responses after thyroxine. That the percentile increase is approximately the same throughout the range of doses of adrenaline employed is shown by the circles. These points correspond to the responses of the lower curve multiplied

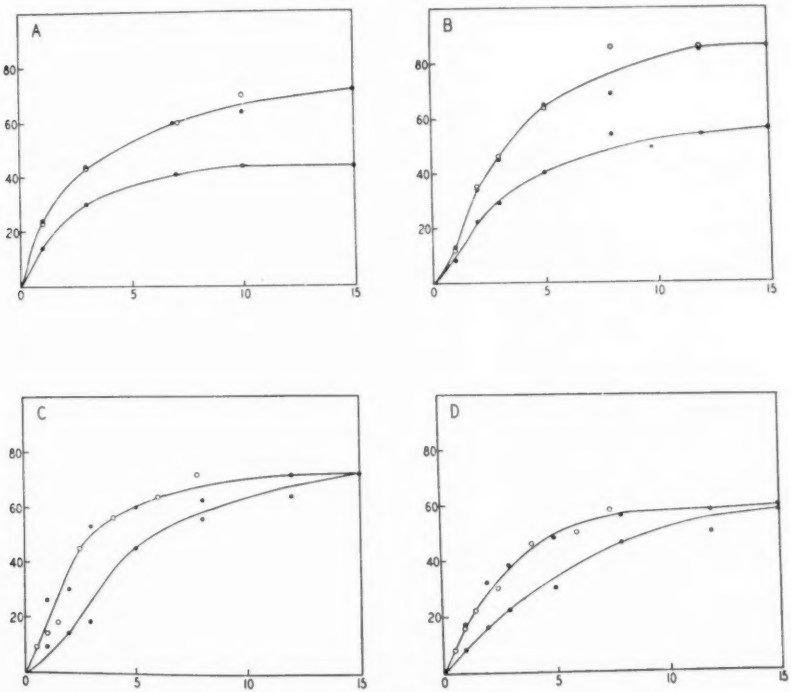

Fig. 3. Differences between the sensitization produced by thyroxine (20 mgm., A and B) and that produced by either cocaine (1 mgm. per kgm., C) or section of the cervical sympathetic (15 days, D). A illustrates the sensitization of the responses to stimulation of the cervical sympathetic at various frequencies; B, C and D, that of the responses to various doses of adrenaline. Ordinates: amplitude (in mm.) of the contractions in the records. Abscissae: in A, frequencies of shocks per second; in B, C and D, doses (γ) of adrenaline. The lower curves are the normal controls. The upper curves (dots) indicate the sensitized responses. The circles were obtained by multiplying the ordinates of the normal responses in A by the factor 1.43, and in B by the factor 1.60. In C and D the abscissae of the normal responses were multiplied by the factor 0.5 to obtain the circles.

by the constant factor 1.60, i.e., the responses to all the doses of adrenaline were approximately 160 per cent greater after thyroxine than before.

This constant ratio of sensitized response to normal response after thyroxine is in striking contrast to the results of sensitization produced by other methods, e.g., by injections of cocaine and by denervation. In figure 3C are shown responses of the membrane to different doses of adrenaline before (lower curve) and after (upper curve, dots) injection of cocaine (1 mgm. per kgm.); and in

figure 3D the influence of severance of the cervical sympathetic (preganglionic denervation) 15 days before the records of the upper curve were made. Although the responses to small doses of adrenaline were clearly increased by these two procedures, those to large doses were practically unaffected. In order to fit the control responses to the curve obtained after sensitization it is necessary to change the scale of the doses of adrenaline, instead of changing the scale of the response, as was done for the thyroxine effect. The circles in figure 3, C and D were obtained by multiplying the abscissae of the normal points by the factor 0.5. The adequate fit obtained after this change of the scale of doses

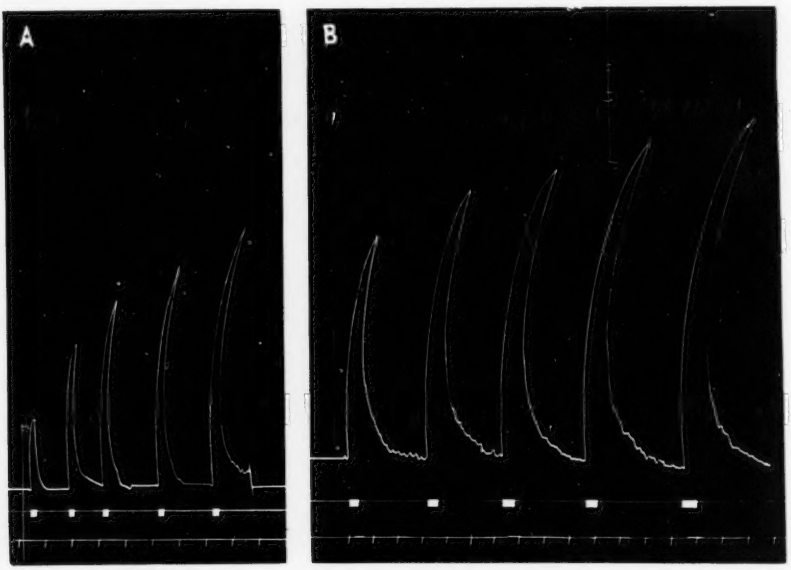

Fig. 4. Increase of the isotonic responses of the nictitating membrane to cervical sympathetic stimulation at frequencies of 1, 3, 7, 10 and 15 per sec. A, control, before thyroxine B, 6 days after administration of 20 mgm. thyroxine.

indicates that any given response was obtained after cocaine or decentralization with one-half the dose necessary in the normal animal.

In all instances the isometric records checked favorably with those obtained isotonically.

B. *Sensitization to stimulation of the cervical sympathetic.* In 7 cats thyroxine sensitized the responses of the nictitating membrane to stimulation of the cervical sympathetic, much as it sensitized the responses to adrenaline. Indeed, a striking parallelism between the two effects was observed (fig. 3, A and B). The optimum dose of thyroxine was the same. The time course of the phenomenon was similar. The degree of sensitization obtained a given day after a given dose of thyroxine was also similar for the two excitatory agents.

In figure 4 are presented typical contractions of the nictitating membrane

from stimulation of the cervical sympathetic before and after thyroxine. In 4A are the normal records from stimulation frequencies of 1, 3, 7, 10 and 15 per second; in 4B, the records on the 6th day after thyroxine (20 mgm.) from the same frequencies and the same intensity of stimulation as in the controls.

C. *Sensitizing action of pituitary thyrotropic hormone.* Subcutaneous injections of the thyrotropic hormone had marked sensitizing action in the 3 normal animals tested. A dose of 0.3 cc. caused a 33 per cent increase of response to adrenaline by the 25th hour. A peak of 39 per cent occurred on the 6th day, and a return to normal within 18 days. Doses of 1.0 cc. were followed by as much as 85 per cent increase in response at the end of 1 hour, but the animals usually died within the next 30 minutes.

As opposed to the results obtained in normal cats, in the 3 thyroidectomized animals studied there was no increase of the contraction of the membrane after the use of the thyrotropic hormone. These negative results were obtained in tests from 2 to 14 days after thyroidectomy.

Discussion. From the above results it is apparent that the sensitizing action of thyroxine on the nictitating membrane is not prompt but delayed. This delay is shown in figure 2. In addition, figure 2 indicates that the action of 10 mgm. of the hormone reaches its peak earlier than that of 20 mgm. and that the duration of the effect is much shorter with the smaller dose, i.e., complete subsidence in 2 weeks as compared with 3 weeks or more.

The effect of thyroxine on the basal metabolic rate (Kunde, 1927) and that on the blood pressure (Krayer and Sato, 1928) is also delayed. In the experiments of Kunde, thyroxine in 10 or 20 mgm. doses caused no elevation of the basal metabolic rate within 7 to 12 hours after it was given. On the second day, however, a maximum of from 13 to 22 per cent increase was attained which declined to normal in 3 to 6 days. The initial delay is not due to the time required for absorption from the alimentary tract because it also occurs after intravenous administration of thyroxine. Thus the time course for the effects of thyroxine on the basal metabolic rate is different from that for its action on the nictitating membrane.

The increased response of the sensitized nictitating membrane over the control is not necessarily dependent on the dose of thyroxine. This is illustrated by figure 2 in which the maximal contractions at the peak with both 10 and 20 mgm. are about the same. However, the peak effects after 20 mgm. appear later than do those after 10 mgm. In addition, when the correct intervals after thyroxine are chosen the percentile increases due to 10 or 20 mgm. may be equivalent. Therefore, the factors which determine the response appear to be the state of the tissues and the time interval after the thyroxine is given.

Severance of the nerve supply to the nictitating membrane (preganglionic denervation) was found to increase the contraction of the membrane to small doses of adrenaline, but the effect with large doses was relatively negligible (fig. 3D). But these decentralized membranes responded to thyroxine like those normally innervated, i.e., after thyroxine the responses resembled those illustrated in figure 3B with an increase to all doses of adrenaline. Here is an in-

stance of a combination of two different types of sensitization, the first due to denervation and the second due to thyroxine.

Not only does thyroxine increase the effects of adrenaline on the nictitating membrane but also those of nerve stimulation. With all frequencies tested the responses were increased (figs. 3A and 4). The degree of increase was independent of the frequency used, if the intensity of stimulation remained the same.

The pituitary thyrotropic hormone causes thyroid secretion and thereby is effective in augmenting the response of the nictitating membrane to adrenaline. Since negative results were obtained with thyroidectomized animals injected with the same substance, it can be inferred that Antuitrin "T" has no direct sensitizing action on the membrane.

The term "sensitization" has usually been defined as a decrease of the dose of a substance required to produce a given response. The sensitization caused by cocaine (Rosenblueth, 1932) or denervation (Cannon and Rosenblueth, 1936) has been attributed to a greater cell permeability or to a slower rate of destruction of adrenaline. Both hypotheses are compatible with the data (fig. 3, C and D). The effects of these two sensitizing procedures agree with the definition of sensitization mentioned above. Since the maximal contractions of the nictitating membrane are not augmented by these types of sensitization, there is no reason to assume that the contractile system of the smooth muscle in the organ has been modified.

Sensitization by thyroxine (fig. 3, A and B), on the other hand, is different from that resulting from cocaine or denervation (fig. 3, C and D). A greater contraction is apparent for all doses of adrenaline and for all degrees of stimulation of the sympathetic supply. These results are better summarized by the statement that the contractile ability of the muscle has been increased rather than by the statement that a weaker stimulation develops a given contraction. It may be concluded that, unlike cocaine or denervation, thyroxine augments the efficacy of the contractile mechanism rather than rendering the nictitating membrane more sensitive.

No data are available as to whether thyroxine produces hypertrophy of smooth muscle, which might account for the increased responses seen. It is interesting, however, to note that thyroxine causes hypertrophy of cardiac muscle (Dock and Lewis, 1932).

SUMMARY

The responses of the nictitating membrane of the cat to injections of adrenaline (figs. 1 and 2) and to stimulation of the cervical sympathetic (figs. 3A and 4) are reversibly increased for some days after an injection of thyroxine (10 to 20 mgm.).

The effect is not immediate; it appears within 20 to 48 hours after the administration; it reaches its peak in 3 to 9 days, and subsides in 14 to 25 days, dependent on the dose given (fig. 2).

The sensitization of response produced by thyroxine differs from that elicited by injection of cocaine or by denervation (fig. 3, C and D). The inference is

drawn that, unlike cocaine or denervation, thyroxine increases the contractility of the membrane (p. 455).

Injections of pituitary thyrotropic hormone cause an increase of the responses of the membrane in normal animals. They have no effect in thyroidectomized animals. It is concluded that this hormone has no direct effect on the membrane, but causes sensitization by the release of thyroid secretion.

I wish to express my thanks to Dr. W. B. Cannon for suggesting this problem, and to Dr. A. Rosenblueth and Dr. G. H. Acheson for their assistance and helpful advice.

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THE EFFECT OF ADRENAL CORTICAL COMPOUNDS ON KETOSIS

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At present, the rôle of the adrenal cortex in the metabolism of fat remains obscure. Although the ketosis following anterior pituitary extract is diminished in the absence of the adrenal glands, the failure of adrenalectomy to abolish the response indicates that pituitary ketogenesis is not exclusively mediated through the adrenal cortex (1). The low order of response in adrenalectomized animals suggests, however, that there is either a partial mediation through the adrenal cortex, or a synergism involving cortical hormone. If a partial mediation were responsible one would expect adrenal cortical hormones themselves to be ketogenic under the same experimental circumstances in which anterior pituitary extract is effective.

The failure of Grollman to produce ketonuria in the rat with a purified adrenal cortical preparation (2) is inconclusive inasmuch as the rat has a high urinary threshold for acetone bodies (3). MacKay and Barnes' positive results (4) were obtained with commercial cortical extract which in itself contains ketone substances capable of producing ketonuria. In the present experiments, cortical preparations were tested by methods which were designed to avoid these two criticisms.

METHODS. Thirty-five male rats of either Sprague Dawley or Yale strain weighing between 120 and 150 grams were used for assay. A group of seven served as controls, while five other groups were injected with Wilson's cortical extract, crystalline corticosterone, desoxycorticosterone, 11 dehydro- 17 hydroxy corticosterone (compound E of Kendall), and crude anterior pituitary extract. The corticosterone and desoxycorticosterone were administered in the form of a fine suspension in 0.9 per cent saline, while compound E was given as a solution in approximately 0.8 cc. of 10 per cent alcohol. There is no reason to suppose that the alcohol which was present in the solution would influence ketosis inasmuch as 1.0 cc. doses of 10 per cent alcohol injected into four rats under similar test conditions were without effect.

The rats were first placed on a fast which was of 18 hours' duration in the case of those receiving compound E, and 48 hours in the remaining animals. A sample of tail blood (0.2 cc.) was taken for analysis, one of the preparations injected, and 4 hours later a second sample of blood was removed. The blood samples were analyzed for total or fractional acetone body content by the micro

blood acetone method previously described (3). The acetone and acetic acid, and beta-hydroxybutyric acid fractions were determined separately in the case of the groups receiving crude cortical extract and anterior pituitary extract. The pituitary extract was prepared by the method of Burn and Ling (5).

In order to demonstrate the effect of crude cortical extract on the urinary excretion of ketone substances, three fed rats were injected with Wilson's extract. Total blood ketones were determined before injection, and again four hours afterward, and the urinary output of these substances during the succeeding 24 hours was determined by the method of Van Slyke (6). The ketone content of the cortical extract was quantitated by the same micro method which was used for the blood analysis.

Results. The failure of adrenal cortical preparations to produce a ketogenic response is quite apparent from the data presented in table 1. Corticosterone, desoxycorticosterone, and compound E were entirely without significant influence on the blood acetone body level. The five animals injected with crude cortical extract likewise did not show a response which could be attributed to a stimulating effect of the extract. The rise in acetone bodies in these animals was confined to the fraction made up of aceto-acetic acid and acetone and was undoubtedly due to the effect of volatile ketones present in the extract. Lot 47 contained 13 mgm. per cc., and lot 768 0.3 mgm. per cc. of volatile ketones. The difference in ketone content of these two preparations is reflected in the difference in blood ketone concentration after their injection. The absence of a rise in beta-hydroxybutyric acid contrasts sharply with the pronounced increase in this fraction in the group receiving a true ketogenic stimulus in the form of a small dose of anterior pituitary extract.

The anomalous ketonuria which is produced by crude cortical extract is revealed by the data presented in table 2. The marked rise in total blood ketones is in itself incongruous inasmuch as a ketonemia of this degree cannot be obtained in the fed rat even with large doses of potent anterior pituitary extract. The ketonuria likewise is distinctly greater than can be produced in fed rats, and here again there are indications that it is not a genuine physiological response, since in the first place the blood acetone concentration did not rise to threshold levels (3), and, moreover, the preformed ketone content is seen to make up from 93 per cent to 97 per cent of the total urinary acetone bodies. In physiological ketosis, this fraction comprises only 20 to 30 per cent of the total.

Discussion. A clue to the identity of the ketones present in commercial cortical extract is offered by Kendall who obtained volatile compounds containing the carbonyl group from cortical extracts when acetone was used for the extraction of the glands. A large part of the volatile material was found to be mesityl oxide (7).

Previous work has shown that in the absence of the adrenal cortex an impairment in ketosis is manifested not only by a lowered sensitivity to anterior pituitary extract (1), but also by a diminished response during exposure to low oxygen tension (8), and after phloridzinization (9). In the light of the present experiment, it is probable that the above decreased responses are attributable to

TABLE 1

The effect of adrenal cortical compounds and anterior pituitary extract on blood acetone bodies

PREPARATION	DOSE	RISE IN TOTAL ACETONE BODIES	PREPARATION	DOSE	RISE IN ACETO-ACETIC + ACETONE	RISE IN BETA HYDROXY	RISE IN TOTAL ACETONE BODIES
	mgm.	*mgm. per cent*			*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
Uninjected controls		+0.3	Compound E (11 dehydro-17 hydroxy-corticos-terone)	2.6 mgm. i.p.			−1.2
		+0.1					+0.3
		+0.2					+1.5
		0.0					+0.9
		+0.8					+0.3
		+2.1					−2.8
		+0.7					−0.6
							−0.8
Average		+0.6					−0.3
Corticosterone	3 s.c.*	−1.5	Cortical extract (Wilson)	3.0 cc. s.c.‡	+7.1	0.0	
	3 s.c.	+1.9		3.0 cc. s.c.§	+3.6	+0.6	
	2 i.p.†	+0.2		3.0 cc. s.c.§	+3.7	+1.0	
	2 s.c.	−1.6		3.0 cc. s.c.§	+1.5	−1.2	
	2 s.c.	+1.2		3.0 cc. s.c.§	+4.8	+0.4	
Average		0.0			+4.1	+0.2	
Desoxycorti-costerone	10 i.p.	+0.2	Anterior pituitary extract	0.5 mgm. solids i.p.	+0.5	+6.4	+6.9
	10 i.p.	+0.1			+0.7	+5.7	+6.4
	10 s.c.	−1.0			+2.1	+5.4	+7.5
	5 s.c.	−0.2			+3.9	+4.2	+8.1
	5 s.c.	0.0			+2.0	+4.8	+6.8
Average		0.0			+1.8	+5.3	+7.1

* Subcutaneously.
† Intraperitoneally.
‡ Lot 47.
§ Lot 768.

Acetone bodies are expressed as acetone and without correction for the 70 per cent conversion of beta-hydroxybutyric acid to acetone.

TABLE 2

Effect of 3 cc. of crude cortical extract (lot 47) on blood and urine ketones of fed rats

BLOOD ACETONE BODIES (MGM. PER CENT)		24 HOUR URINARY EXCRETION (MGM.)	
Before injection	4 hours after injection	Acetone plus aceto-acetic acid	Beta-hydroxybutyric acid
1.7	11.1	3.4	0.2
1.5	11.1	3.8	0.3
1.7	11.8	3.2	0.2

a lack of normal synergism supplied by cortical hormone rather than to a loss of any direct ketogenic effect. Likewise, the restoration of acetone body excretion in diabetic adrenalectomized animals by cortical hormone (9) most likely involves a supportive action of the hormone in which a mechanism of ketosis is facilitated rather than initiated. This is in contrast to the action of the hormone in carbohydrate metabolism where it exerts a direct influence on glycosuria (10, 11) and on tissue glycogen (12).

CONCLUSIONS

Adrenal cortical extract, corticosterone, desoxycorticosterone, and 11 dehydro-17 hydroxy corticosterone (compound E of Kendall) do not stimulate ketosis in the fasting rat.

We are indebted to Dr. E. C. Kendall for the corticosterone and 11 dehydro-17 hydroxy corticosterone used in these experiments. The desoxycorticosterone was supplied by the Schering Corporation through the kindness of Dr. W. H. Stoner, and the cortical extract by the Wilson Laboratories through the courtesy of Dr. David Klein.

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THE ACTION OF THIAMINE AND COCARBOXYLASE UPON THE FROG VENTRICLE

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The most striking symptoms of vitamin B_1 deficiency in animals involve changes in the function of the heart and of peripheral neuromuscular systems. The immediate origins of these effects are still unknown. To some extent they may be due to central nervous changes, such as the lowered respiratory rates demonstrated by Peters et al. in the brain tissue of deficient pigeons and rats in the presence of certain substrates (Gavrilescu and Peters, 1931; O'Brien and Peters, 1935). The possibility of more direct, local effects upon peripheral systems has scarcely been explored. Myelin sheath degeneration is known to be characteristic of advanced deficiency; but this may be preceded by functional impairment, and conversely deficient animals may recover functionally on treatment with thiamine long before histological repair can be detected (Williams and Spies, 1938).

These considerations suggest that thiamine may play some direct part in neuromuscular function. Three independent developments indicate such a relationship. Minz et al. have reported *a*, that thiamine enhances the action of acetylcholine on isolated leech muscle and rat intestine and on the arterial pressure of the cat; and *b*, that thiamine is liberated in the activity of the vagus nerve (Agid, Beauvallet and Minz, 1937; Minz and Agid, 1937; Minz, 1938). Glick and Antopol (1939) have shown that the action of choline esterase *in vitro* may be inhibited up to 50 per cent with thiamine in concentrations of about 10^{-3} by weight, indicating that thiamine may exert effects comparable with those of eserine. Finally Mann and Quastel (1940) have reported that brain slices from polyneuritic pigeons synthesize acetylcholine at subnormal rates in the presence of pyruvate and high concentrations of phosphate, and that the rates may be restored to normal by the addition of thiamine *in vitro*. It is noteworthy that all these reports indicate activities of thiamine which *enhance* those of acetylcholine.

The present paper is concerned with the action of thiamine and of thiamine pyrophosphate (cocarboxylase)[2], alone and in combination with acetylcholine,

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[2] The cocarboxylase employed in these experiments is a synthetic crystalline preparation containing about 60 per cent thiamine pyrophosphate. The remainder consists primarily of the monophosphate ester.

upon the Straub preparation of the frog heart. In this preparation test solutions act directly upon the ventricular muscle, without ordinarily entering the auricles or reaching the cardiac pacemaker. For this reason only changes in the amplitude of the beat, not in its frequency, can ordinarily be observed.

Previous experiments with this preparation have led to confused results. Hecht and Weese (1937) reported that thiamine 10^{-2} by weight has no effect on the heart. Agid and Balkanyi (1938), however, reported that at concentrations of 10^{-7} to 10^{-5} the amplitude of the beat was decreased. With increase in concentration this effect declined and even reversed, and as the concentration approached 10^{-2} a second reversal of effect occurred, resulting in heart block. These actions were transient and were inhibited by atropine. Furthermore, vitamin B_1 was found to antagonize the action of acetylcholine, so that, for example, an arresting dose of the latter could reinstate the beat in a heart previously blocked with 10^{-2} thiamine.

Kaiser (1939) clarified this situation in part with the reminder that thiamine in its usual marketed form—the chloride hydrochloride—is highly acidic. In unbuffered or lightly buffered Ringer solutions it yields pH's of 3.5–4.5. Kaiser reported that neutral solutions of thiamine in concentrations of 10^{-7} to 10^{-2} have no effect upon the Straub heart. The inhibitory action of acid thiamine can be reproduced with acid Ringer alone; and unlike that of acetylcholine, this effect is not inhibited by atropine. On the other hand, the neutral vitamin antagonizes the action of acetylcholine when applied to the heart simultaneously with it, an effect comparable with that reported by Agid and Balkanyi.

The present experiments confirm and extend Kaiser's observations. They show that thiamine exerts two independent effects upon the frog ventricle: 1, a specific strong depressant action in slightly acid solutions (pH 5.2–6.0) at concentrations of 10^{-4} to 10^{-3} by weight. Acid Ringer exerts comparable effects only at much lower pH's. This action is exhibited also by cocarboxylase 10^{-3} in acid, and to a slight degree even in neutral solutions. None of these activities is inhibited by atropine. 2. In neutral solutions thiamine 10^{-5} to 10^{-3} antagonizes the depressant action of acetylcholine when applied to the heart simultaneously with it. This effect begins at pH's at which the direct depressant action of thiamine ceases, and increases sharply with further increase in alkalinity (pH 5.9–7.6). It is not exhibited by cocarboxylase 10^{-3} under any observed circumstances.

The action of thiamine—or of cocarboxylase—upon the Straub preparation therefore in no case enhances that of acetylcholine. Under some circumstances it passively adds to, and otherwise it antagonizes, that of the latter substance.

METHODS. All experiments were performed with the Straub preparation of the isolated heart of *Rana pipiens* at constant temperatures. Both the amplitude and frequency of the beat were recorded kymographically. Following assembly of the preparation the heart was permitted to come to a steady state during 45 to 60 minutes undisturbed in bicarbonate Ringer I (table 1). Test solutions were then applied at regular intervals and for regular durations, in-

terpolated with standardized periods of washing with Ringer I. During perfusions oxygen was constantly bubbled through the solutions. The test solutions were made up in bicarbonate-free Ringer II (table 1). Their pH was adjusted with NaOH or HCl. All pH measurements were performed with a Beckman glass-electrode meter.

In our early experiments a standard Straub cannula was employed. This was emptied and refilled with capillary pipettes, with care that the height of the perfusion fluid remained constant throughout the experiment.

It was found, however, that the heart rapidly neutralized the small volumes (1 ml.) of acid solution used in the perfusions. To eliminate this discrepancy a continuous-flow cannula was designed in which the height of the perfusion fluid was kept constant by means of an overflow, and through which solutions were run at the rate of about 13 ml. per minute. Under these conditions the pH of the test mixtures remained constant within 0.02 pH unit throughout each experiment.

TABLE 1

RINGER I		RINGER II	
NaCl	0.65 gram	NaCl	0.65 gram
KCl	0.014	KCl	0.014
$CaCl_2$	0.012	$CaCl_2$	0.012
$NaHCO_3$	0.02	KH_2PO_4	0.005
NaH_2PO_4	0.001	Na_2HPO_4	0.005
H_2O	to 100 ml.	H_2O	to 100 ml.
Normal pH	7.8	Normal pH	6.45

Observations. *The depressant actions of thiamine and cocarboxylase.* A solution of thiamine chloride hydrochloride 10^{-3} in Ringer II has a pH of 4.5. Such a solution immediately blocks the beat of the ventricle in diastole. The auricle may continue to beat, and frequently fills while the heart is in this condition, permitting the test solution to bathe structures which it otherwise does not reach. This may account for certain irregularities—resumption and periodic waxing and waning of the beat ("Luciani periods")—sometimes encountered in later stages of this experiment. On replacing the thiamine solution with Ringer I the ventricular beat recovers immediately, and attains its original amplitude within a few seconds. Cocarboxylase 10^{-3} in Ringer II, brought to pH 4.5, behaves similarly to thiamine. Ringer II alone at pH 4.5, however, depresses the amplitude of the beat comparatively little (fig. 1). In solutions of this acidity, therefore, thiamine and cocarboxylase specifically inhibit the amplitude of the ventricular beat. This effect was examined further in the following experiments.

A first series was conducted in April to June, 1940, at a temperature of 13.5 to 14.5°C., using the ordinary Straub cannula. Except in cases of complete arrest of the beat, test solutions were allowed to act for three minutes, and were applied

at 10 minute intervals. Between tests the heart was allowed to beat in several changes of Ringer I. Ten such preparations were studied. In every case to produce comparable depressions of the beat amplitude, Ringer II alone had to be brought to a full pH unit or more below solutions of Ringer II containing 10^{-3} thiamine (fig. 2A).

As noted above, during these perfusions with the ordinary cannula, the pH's of the test solutions shifted rapidly toward neutrality. After 3 minutes' contact

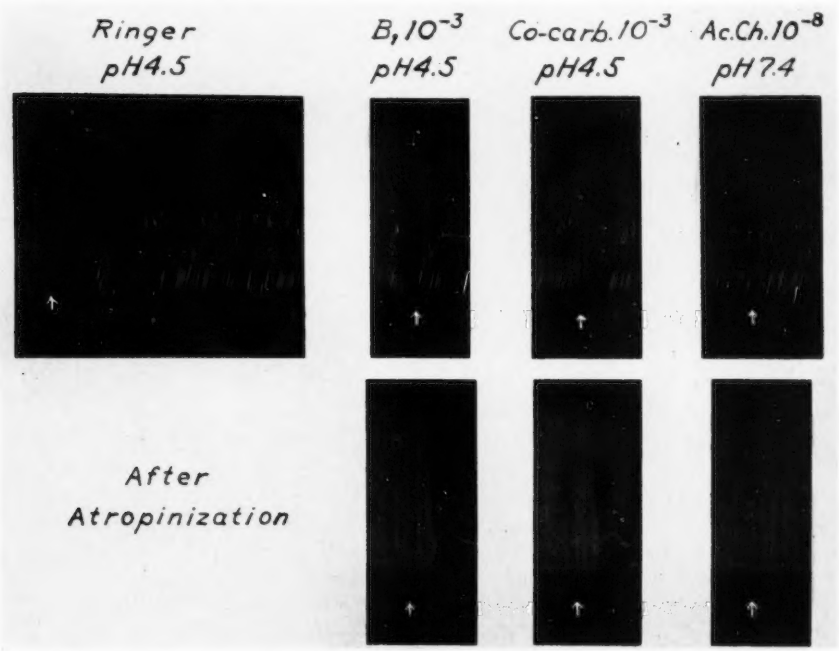

Fig. 1. Kymograms of the ventricular beat before and after atropinization (upper and lower series respectively). All records were made with a single heart preparation, using the flow cannula. At pH 4.5 Ringer solution depresses the beat slightly, thiamine (B_1) and cocarboxylase 10^{-3} stop it completely. After atropinization the action of thiamine or cocarboxylase is unchanged, while that of acetylcholine is abolished. The time signal at bottom marks tenths of a minute. A white arrow on each record indicates the moment of application of the test solution. The lower portion of the tracing represents diastole.

with the heart, the pH of acid Ringer solutions had changed as much as 2 units (e.g., from 4 to 6); under similar conditions the pH of solutions containing thiamine changed much less (usually 0.2 0.5 unit). The results obtained by this method therefore were unnecessarily scattered and misleading.

A comparable series of experiments was performed with the constant-flow cannula at 17°C. in June to August, 1941. Test solutions were applied to the heart for 10 minute intervals, separated by 30 minute periods in Ringer I alone. Twelve preparations were examined; the results are collected in figure 2B.

Since in these experiments the heart was given no opportunity to neutralize the test solutions, the effects occur at considerably higher pH's than when the ordinary cannula was used. For the same reason they are much more uniform. Acid Ringer alone inhibits the heart progressively from pH 5.2 to complete block at about pH 4; thiamine 10^{-3} in Ringer produces comparable effects in the pH range 6 to 5.2; that is, the depression due to thiamine is complete at a pH at which Ringer alone produces no effect whatever.[3]

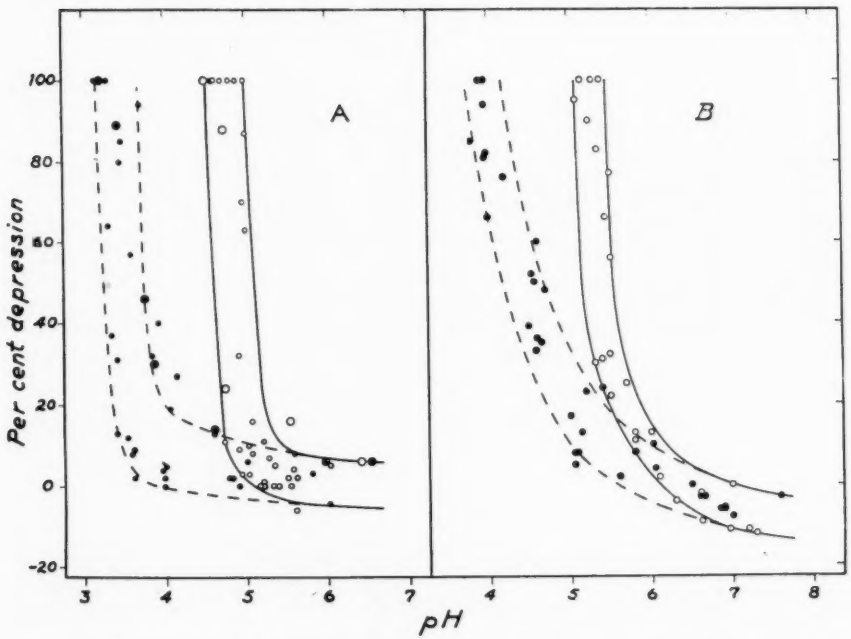

Fig. 2. Depression of the amplitude of the ventricular beat with Ringer solution alone (solid circles) and with thiamine 10^{-3} in Ringer (open circles) at various pH's. *A*—Experiments with the ordinary cannula (10 preparations); the pH stated is that of the test solution before use, but this changed in contact with the heart. The large circles show data from a single preparation. *B*—Experiments with the flow cannula at constant pH (12 preparations).

At a concentration of 10^{-4} thiamine still exhibits this specific effect, though to a much smaller degree. Thiamine 10^{-5} produces very nearly the same effect as Ringer II alone at the same pH. Thus in an experiment at pH 4.6, thiamine 10^{-3} stopped the beat, thiamine 10^{-4} depressed its amplitude 49 per cent, thiamine 10^{-5} 19 per cent, and Ringer II alone 13 per cent.

Between pH 6 and 7.6, at which thiamine exhibits no depressant action,

[3] In a preparation blocked with either acid Ringer or thiamine the ventricle does not respond to direct electrical stimulation.

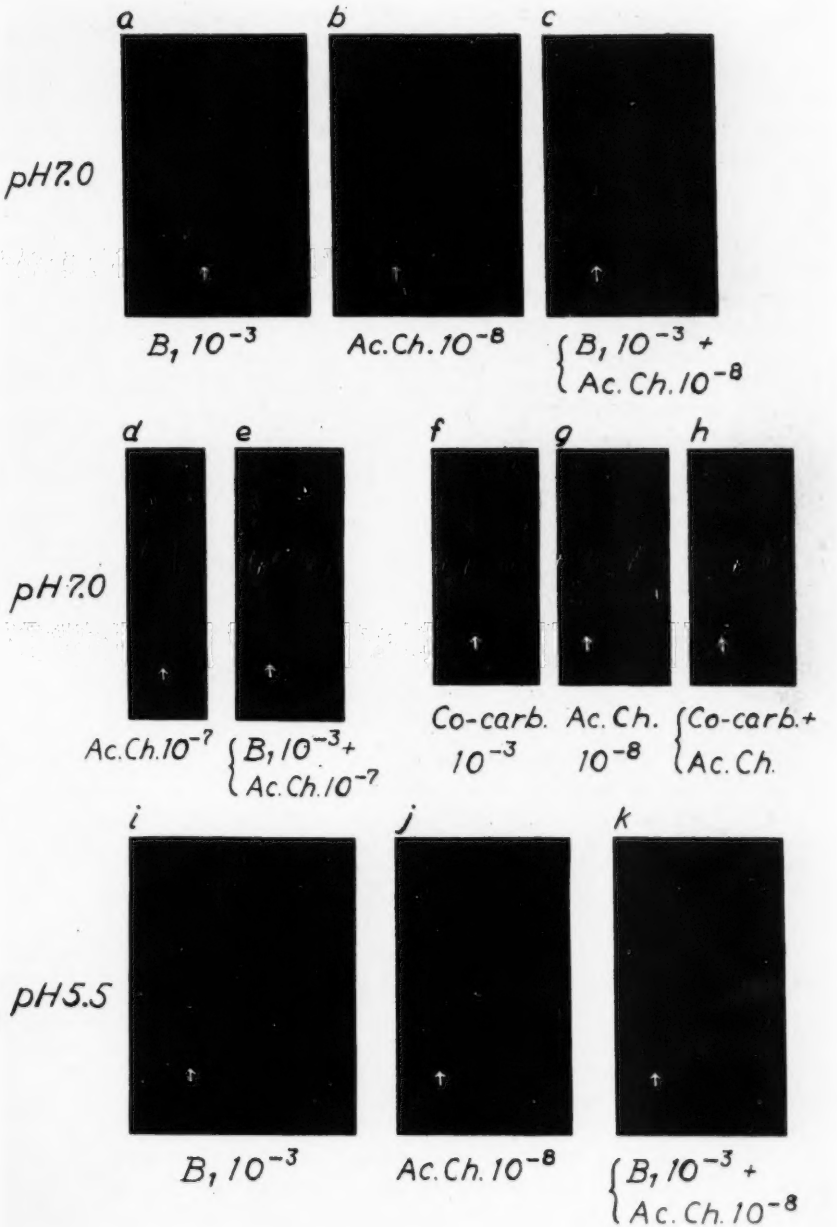

Fig. 3—*a-e*. The antagonism of thiamine (B_1) 10^{-3} to the action of acetylcholine 10^{-8} and 10^{-7} at pH 7.0. *f-h*. At the same pH the depressions due to cocarboxylase and acetylcholine are additive. *i-k*. The depressions due to thiamine and acetylcholine at pH 5.5 also are additive. Records *a-e* and *i-k* were made with a single heart preparation, *f-h* with another. Signals as in figure 1.

cocarboxylase 10^{-3} still depresses the amplitude of the beat 10 to 25 per cent (fig. 3f). Thiamine solutions or Ringer II alone above pH 6.5 cause small increases in the beat amplitude. These are identical in the presence or absence of thiamine, and appear to be due to the pH itself (fig. 2B).

The depressant effects of acid solutions, of thiamine, and of cocarboxylase, unlike those of acetylcholine, are not inhibited by atropinization. In the experiment shown in figure 1, after the upper series of tracings had been recorded, the heart was allowed to beat for 15 minutes in atropine sulphate 10^{-5} at pH 7.5. It was then washed for 5 minutes in Ringer I. On subsequent reapplication of the test solutions, the actions of thiamine and of cocarboxylase were found to be unaffected, while those of acetylcholine 10^{-8} had been completely abolished. This is an initial indication that the depressant effects of thiamine and cocarboxylase are not mediated through the acetylcholine system.

TABLE 2

Interaction of thiamine and cocarboxylase with acetylcholine

In neutral solution the effects of thiamine and acetylcholine are antagonistic. In acid solution they are additive; and the same is true of cocarboxylase and acetylcholine in neutral solution. The numbers in parentheses indicate concentrations by weight.

pH	PERCENTAGE DEPRESSION					HEART NUMBER
	Ac. Ch.	Thiamine	Ac. Ch. + thiamine	Cocarb. 10^{-3}	Ac. Ch. + cocarb.	
5.5	(10^{-8}) 68	(10^{-3}) 69	89			1
6.95	(10^{-8}) 54	(10^{-6}) 0	51			2
6.95	(10^{-8}) 54	(10^{-5}) 0	48			2
6.95	(10^{-8}) 54	(10^{-4}) 0	20			2
6.95	(10^{-8}) 54	(10^{-3}) 0	9	—	65	2
7.0	(10^{-8}) 42	(10^{-3}) 0	13	27	56	1
7.0	(10^{-7}) 100	(10^{-3}) 0	24			3
7.55	(10^{-7}) 45	(10^{-3}) 0	11	10	47	4

The antagonism of thiamine and acetylcholine. Thiamine 10^{-3} in neutral solution, which itself has no effect upon the Straub preparation, decreases markedly the action of acetylcholine when both substances are applied to the heart simultaneously (fig. 3 a–e). In this sense thiamine exhibits an activity comparable with that of atropine. Unlike atropinization, however, the thiamine effect is washed out of the heart with great ease and rapidity. After a heart has beaten in neutral thiamine 10^{-3} for as long as an hour, a 5 minute washing with Ringer I completely abolishes all effect upon the action of acetylcholine applied subsequently (cf. Kaiser, 1939). A large antagonism to acetylcholine is still exhibited by thiamine at a concentration of 10^{-4}; at 10^{-5} the effect has become very small, and below this concentration it is negligible (table 2).

The antagonism to acetylcholine is exhibited only at pH's above about 5.9 at which the specific depressant action of thiamine has ceased. With increase in pH above 5.9 acetylcholine antagonism increases sharply; below 5.9 thiamine and acetylcholine applied together to the heart simply add their individual de-

pressant effects (fig. 3, i–k). To state this in another way, thiamine in the presence of acetylcholine reverses its action on the heart muscle at pH 5.9: below this pH it is a specific depressant, above it, it increases the amplitude of the beat by antagonizing the depressant effects of acetylcholine.

On the other hand cocarboxylase, which depresses the heart in both acid and neutral solution, does not antagonize the action of acetylcholine at either reaction; in all cases the effect of adding the two substances simultaneously is simply additive (fig. 3, f–h; table 2). We therefore have the extraordinary result that these two effects upon the frog ventricle are mutually exclusive: at pH's at which thiamine antagonizes acetylcholine it does not depress the beat, and *vice versa*; while cocarboxylase, which depresses the beat under all observed circumstances, does not antagonize acetylcholine activity in any of them.

The separation between these phenomena is complete. Depression of the heartbeat with acid thiamine or with cocarboxylase adds simply and quantitatively to that produced by acetylcholine. For example (table 2), thiamine 10^{-3} at pH 5.5 cuts the amplitude of the beat to 31 per cent (69 per cent depression); acetylcholine 10^{-8} cuts it to 32 per cent; both substances together to 11 per cent. The expected amplitude, if both substances were acting additively, would be 0.31 x 0.32 or 10 per cent. Similarly cocarboxylase and acetylcholine acting additively at pH 7.0 should yield an amplitude of 42 per cent; an amplitude of 44 per cent is observed. At pH 7.55 the expected amplitude for both substances acting together is 50 per cent, the observed amplitude 53 per cent. It may be concluded that there is no specific interaction whatever between acetylcholine and neutral cocarboxylase or thiamine in solutions more acid than pH 5.9.

Certain collateral information has implied that thiamine or cocarboxylase might depress the heartbeat through the intermediation of acetylcholine, for example through inhibition of choline esterase (Glick and Antopol, 1939), or stimulation of acetylcholine synthesis (Mann and Quastel, 1940). The foregoing observations preclude such a relationship. In this they are consistent with the observed failure of atropine to inhibit thiamine or cocarboxylase activity.

Discussion. The depressant actions of thiamine and cocarboxylase upon the frog ventricle are independent of that of acetylcholine. The only type of interaction with acetylcholine which has been demonstrated—that of thiamine in neutral solution—is an antagonism. It is opposite in direction therefore to other types of interaction previously reported, all of which tend to further acetylcholine activity: the acetylcholine-enhancing effect of Minz et al.; the eserine-like inhibition of choline esterase *in vitro* (Glick and Antopol, 1939); and the stimulation of acetylcholine synthesis by thiamine in the polyneuritic brain (Mann and Quastel, 1940). These mechanisms may operate in other structures of the neuromuscular system. Clearly none of them is applicable to the behavior of the present preparation.

The sharp transition between the depressant action of thiamine and its antagonism to acetylcholine at pH about 6 may be related to the state of dissociation of the molecule. Below pH 6 the free amino group of thiamine progressively

adds a proton (H^+) to form the doubly charged cation thiamine^{++}; it is in this form that it exists as the chloride hydrochloride. This change is half completed at pH about 5 (Williams and Ruehle, 1935; Williams, 1937). Between pH 6 and 8 thiamine exists entirely in the form of the monovalent ion, thiamine$^+$, and possesses a free amino group. It is possible that the depressant action of thiamine is associated with the first state of the molecule, acetylcholine antagonism with the second.

The diffusion into cells of a large molecule like thiamine, though much more rapid than that of cocarboxylase, must still be a relatively slow process (Banga, Ochoa and Peters, 1939). Yet both substances depress the heartbeat almost instantly, thiamine not appreciably faster than cocarboxylase; and conversely this effect is obliterated by washing with Ringer solution within a few seconds. Similarly, effects antagonistic to the action of acetylcholine are washed out of the heart with great speed even after long periods of soaking in thiamine solution. It is difficult to avoid the conclusion that both types of action occur at the muscle surface.

It may be asked whether the observed phenomena have any significance in the behavior of the intact organism. This question is intimately bound up with the nature of the Straub preparation, in which the ventricle is bathed in test fluids much in the same way that it is bathed in situ with the blood, while the cardiac pacemaker is left unaffected.

Cocarboxylase is found in the blood almost entirely within the cells. The free thiamine concentration of normal human plasma is about 10^{-8} by weight; that of cattle and pigeons is of the same order (Goodhart and Sinclair, 1939). At this concentration thiamine produces no perceptible effect upon the frog ventricle. The gross concentration of thiamine in animal muscles is of the order of 10^{-6} by weight; such concentrations in the perfusion medium of our experiments also were without effect. The significance of the present phenomena *in vivo* rests therefore upon the degree to which thiamine and cocarboxylase may be concentrated at specific loci, perhaps for limited intervals, within the circulatory and neuromuscular systems. No basis yet exists for evaluating this factor.

SUMMARY

Experiments are reported upon the action of thiamine (vitamin B_1) and of its pyrophosphate ester (cocarboxylase) upon the Straub preparation of the frog heart. This is a ventricular preparation, in which test solutions act directly upon the heart muscle and produce changes only in the amplitude of the beat.

Between pH 6.0 and 5.2 thiamine 10^{-3} by weight progressively depresses the beat to complete stoppage. At a concentration of 10^{-4} this effect is still observed, at 10^{-5} it has become negligible. Cocarboxylase 10^{-3} behaves similarly, but continues to depress the beat 10 to 25 per cent up to pH 7.6.

This action is independent of that of acetylcholine. Atropine, which completely abolishes the latter, has no effect upon the former. Further, the depressions produced by thiamine or cocarboxylase and by acetylcholine are simply

and accurately summated when both types of substance are applied to the heart simultaneously.

Above pH about 6.0 thiamine 10^{-5} to 10^{-3} progressively antagonizes the action of acetylcholine. This effect is not exhibited by cocarboxylase. Depression of the heartbeat and acetylcholine antagonism therefore appear in these experiments under mutually exclusive conditions: thiamine shifts from the first to the second type of activity at pH about 6, while cocarboxylase, which depresses the beat at all pH's investigated, does not antagonize acetylcholine at all. The shift in behavior of thiamine with pH may be related to its change from a monoacidic to a diacidic base at pH's below 6. The significance of these effects in the intact animal can not yet be evaluated.

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INCREASED REQUIREMENTS OF PANTOTHENIC ACID AND VITAMIN B_6 DURING EXPERIMENTAL HYPERTHYROIDISM[1, 2]

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Various studies have been made on the effect of hyperthyroidism on vitamin metabolism and vitamin requirements. In 1932 Himwich et al. (4) demonstrated an increased requirement for undifferentiated vitamin B during thyroid feeding. Sure and Buchanan (6) reported that the requirement of the rat for crystalline vitamin B_1 was increased during hyperthyroidism. It has also been shown that thyroid feeding decreases the body stores of vitamin B_1 (2, 5).

Drill and Sherwood (3) found that rats fed a normal diet, plus sufficient thyroid gland to produce a loss in weight, would stop losing weight when vitamin B_1 was injected, but would not regain their lost weight until a supplement rich in the vitamin B_2 complex was added to their diet. This indicated that some B vitamins other than thiamin were needed in increased amounts during experimental hyperthyroidism. A study was therefore made, using crystalline B vitamins, to determine which vitamin(s) of the B_2 complex are required in larger amounts during thyroid feeding.

METHODS. Female rats averaging 200 grams in weight were used as experimental animals. A sex difference in response to thyroid feeding in rats has been reported (1). The rats were fed the following diet (diet no. 8) ad libitum: salts, 4; cod liver oil, U.S.P., 4; Crisco, 10; casein, 20; starch, 62 parts. Each rat also received a daily supplement of 200 mgm. of a dried baker's yeast. The yeast contained 29 International Units of vitamin B_1 per gram and 23 Sherman-Borquin units of vitamin G (flavin) per gram. The thyroid gland contained 0.21 per cent iodine.[3] Water was constantly supplied.

RESULTS. Nine normal rats, receiving diet 8 plus the yeast supplement, gained weight during the experiment (fig. 1). Nine rats, serving as negative controls, received diet 8 ad libitum and the daily yeast supplement plus 100 mgm. of thyroid gland per day. These rats showed a progressive loss of weight (fig. 1) and were killed on the 47th day of the experiment. Twenty-three test

[1] The authors wish to thank Eli Lilly and Company for a grant in support of this work.

[2] This work was done in the laboratory of Dr. W. W. Swingle and we are indebted to him for the necessary facilities to undertake this work.

[3] The authors are indebted to Dr. C. N. Fry of The Fleischmann Laboratories for supplying the analysed yeast, to Dr. R. T. Major of Merck and Company for supplying the crystalline B vitamins, and to Dr. H. W. Rhodehamel of Eli Lilly and Company for the desiccated thyroid gland.

animals, receiving the same diet and supplements as the negative controls, also lost weight. On the twentieth day of thyroid feeding the test rats had lost an average of 24.4 grams of body weight. They were then injected with 500 gamma of thiamin (vitamin B_1) per day and the loss of weight was stopped. The thiamin injections were continued for 11 days during which time the average weight of the group remained constant, neither gaining nor losing, as previously reported (1, 3, 5).

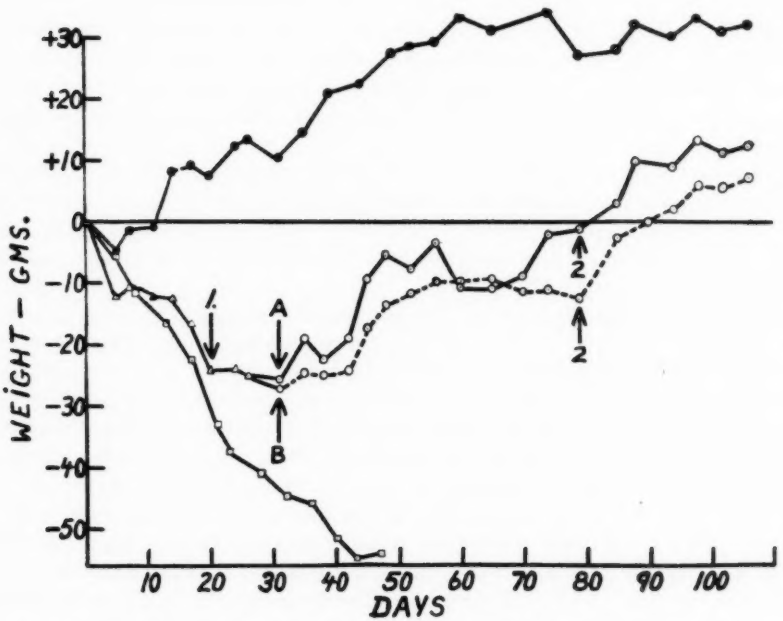

Fig. 1. Average change in weight of the rats. Control rats on diet 8 ●———●. Negative controls fed diet 8 plus 100 mgm. of thyroid gland per day ⊡———⊡. Test animals fed diet 8 plus 100 mgm. of thyroid gland per day ◬———◬, with supplements added at points indicated. *1*, test animals injected with 500 gamma of thiamin per day. Test animals divided into group A ⊙———⊙ and group B ⊙---------⊙. Group A injected with 500 gamma of each pyroxidin and thiamin per day. Group B injected with 1 mgm. of calcium pantothenate and 500 gamma of thiamin per day. *2*, groups A and B injected with 1 mgm. of calcium pantothenate and 500 gamma of each pyroxidin and thiamin per day.

At this point the test animals can be made to gain weight if a rich source of the vitamin B_2 complex is added to the diet (3). However, on the 31st day of thyroid feeding the test rats were divided into two groups, A and B. Group A received 500 gamma of pyroxidin (vitamin B_6) per day in addition to the vitamin B_1 being injected. The pyroxidin and thiamin were dissolved in one solution and brought to pH 6.8, so that 0.5 cc. contained 500 gamma of each pyroxidin and thiamin. These rats made a rapid gain in weight (fig. 1). Group B

was injected with 0.5 cc. per day of a solution containing 500 gamma of thiamin and 1 mgm. of calcium pantothenate. This group definitely gained weight, but at a slower rate than the rats receiving the pyroxidin injections.

Group A, receiving the pyroxidin and thiamin injections, had nearly reached its original weight on the 79th day of the experiment. Group B, however, receiving the thiamin and calcium pantothenate injections, did not gain weight to their original level in 79 days, but reached a constant level about 11 grams below their initial weight (fig. 1). On the 79th day of thyroid feeding groups A and B received a daily 0.5 cc. injection of a solution containing 500 gamma of thiamin, 500 gamma of pyroxidin, and 1 mgm. of calcium pantothenate. Both groups now gained weight rapidly, the average weight being above the weight at the start of thyroid feeding. The injections and the thyroid feeding were continued until the 106th day, during which time the increase in weight was maintained.

Discussion. Vitamins A and C are required in larger amounts during hyperthyroidism but do not influence the loss of weight as do the B vitamins. The diet used in the above experiment contained 4 per cent cod liver oil. If 2 per cent of cod liver oil is used some of the thyroid fed rats will develop xerophthalmia.

The diet was planned to contain a minimal but adequate amount of yeast, as evidenced by the gain in weight of the untreated animals (fig. 1). The amount of thyroid gland fed was sufficient to produce a loss of weight with this normal diet. Thus, any increase in the body requirement for the B vitamins, caused by thyroid feeding, will result in a relative deficiency of these vitamins.

When thyroid feeding was started the rats showed a progressive loss of weight. Thiamin is known to be required in larger amounts during hyperthyroidism and its injection when the rats had lost 24.4 grams in weight stopped any further loss of weight. The food intake was simultaneously increased from a normal of 11 to 12 grams per day, by the thiamin injections, to approximately 17 to 19 grams per day. This increase in food intake seems to be the main factor in stopping a further loss of weight, even while thyroid gland is still being fed. It has been shown that the addition of a rich source of the vitamin B_2 complex (as yeast) at this time would enable the thyroid fed rats to regain their lost weight without, however, further increasing the food intake (3). Thus, the effect of the yeast is probably through its additional content of some vitamins in the B_2 complex, as thiamin was already being injected in an optimal amount. It was found that injections of both pyroxidin and calcium pantothenate would enable the rats to regain their lost weight, and would effectively replace the addition of yeast. Pyroxidin injections caused a more rapid gain in weight than calcium pantothenate alone. When both pyroxidin and calcium pantothenate were injected together the rats regained all of their lost weight. The gain in weight was maintained, while still feeding thyroid gland. It was not necessary to supplement the diet with riboflavin. It is evident that the requirements for three B vitamins, thiamin, pyroxidin, and pantothenic acid are increased during experimental hyperthyroidism.

CONCLUSIONS

Rats fed a diet containing a normal but minimal amount of yeast lost weight when thyroid gland was fed. Injections of thiamin will stop the loss of weight, but the rats will not regain their lost weight until a rich source of the vitamin B_2 complex, as yeast, is added to the diet. Injections of pyroxidin (vitamin B_6) and calcium pantothenate can effectively replace this addition of yeast, and the rats regained and maintained their lost weight while still receiving thyroid gland. Thus in addition to thiamin, both pyroxidin and pantothenic acid are required in larger amounts during experimental hyperthyroidism.

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ADDENDUM

Mr. R. F. Light of the Fleischmann Laboratories has informed us that the yeast supplement also contained 85 gamma of vitamin B_6 and 120 to 150 gamma of calcium pantothenate per gram.

IN VIVO AND IN VITRO EXCHANGE OF PHOSPHORUS BY ENAMEL AND DENTIN[1]

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Chievitz and Hevesy (1) and Hevesy, Holst and Krogh (2), using radioactive phosphorus as the indicator, compared the rates of exchange of phosphorus by whole teeth and other tissues in the case of a variety of animals. Manly and Bale (3) demonstrated that the labeled phosphorus acquired *in vivo* by calcified tissues enters the mineral material. Hevesy and Armstrong (4) found, after the parenteral administration of potent preparations of radioactive phosphorus to mature cats, small but significant amounts of the labeled isotope in the enamel and much larger quantities in the dentin. Volker and Sognnaes (5) also demonstrated an uptake of radiophosphorus by the enamel of cats. However, Manly, Hodge and Van Voorhis (6) failed to confirm an exchange of phosphorus by the enamel but, as they stated, their result might have been a consequence of the administration to the animal of a preparation of radiophosphorus too weak to activate the enamel to a measurable degree. These workers found that the active phosphorus content of the dentin was constant between various teeth, and of the same order as that of dense diaphyseal bone. While the work to be reported in this paper was in progress, Sognnaes and Volker (7) described the results of a study of the distribution of radiophosphorus in the enamel of experimental animals and presented evidence that the greatest amount of the active isotope found in the surface layer of enamel was derived from the saliva.

Because the results of Manly, Hodge and Van Voorhis (6) differed from those of Hevesy and Armstrong (4), it seemed necessary to determine more precisely the relative *in vivo* uptakes of radioactive phosphorus by enamel and by dentin. This purpose has been accomplished by the use of a very potent preparation of radioactive phosphorus and by the measurement of the activities of the separated enamel and dentin under identical conditions. Additional information was obtained as to the degree of phosphorus exchange by dental structures and as to the routes over which the exchange occurs. While it does not seem probable that the radioactivity found by Hevesy and Armstrong (4) in enamel could have been due entirely to contamination of the enamel specimens with dentin, the

[1] This work was made possible by a grant from the Carnegie Corporation and was aided by the personnel of the Works Progress Administration, Official Project no. 665-71-3-69, Sub-project no. 344. The active phosphorus used in this investigation was kindly supplied by the Radiation Laboratory of the University of California.

improvement, made by Manly and Hodge (8), of the Brekhus and Armstrong (9) method for the separation of enamel and dentin allowed this point to be reinvestigated. This procedure (8) is based upon the suspension of the finely powdered whole tooth in an inert liquid of density between those of enamel and dentin, whereupon, after centrifugation, the enamel sinks and the lighter particles of dentin float at the surface of the liquid. In order to determine the influence of the possible presence of dentin on the radioactivity of the initial enamel specimen, the activity of the enamel was measured after each of two repurifications, each repurification being made from a heavier liquid than that employed in the preceding stage. The enamel of four animals was pooled and mixed in order that sufficient material would be available for this experiment.

Four mature cats (weight 7.0–9.2 lbs.) were given by subcutaneous injection a sodium phosphate solution, adjusted to pH 7.4 containing 23.6 mgm. total phosphorus and 32×10^6 counts per minute of the radioactive isotope of phosphorus. The solution was distributed among the animals in proportion to the $\frac{2}{3}$ power of their weight. The animals were sacrificed 122 hours after the injection, when their teeth and a femur from each animal were obtained and a sample of blood drawn from the heart of each animal. The teeth were cleaned and the roots covered with colored collodion, after which they were washed for 24 hours in running water, and for 24 hours in a phosphate solution containing 10 mgm. of the inactive isotope of phosphorus per 100 cc. The crowns of the teeth were cut off the roots, cracked into small bits and extracted over night with an equal part mixture of alcohol and ether. They were then pulverized so that all material passed through a 100-mesh screen and the enamel and dentin separated by the procedure of Manly and Hodge (8). The enamel which repeatedly sank from a bromoform-acetone mixture of density 2.70, and the dentin which deposited no material from a liquid of density 2.42 but which sank from one of density 2.08, were the initial specimens whose radioactivities were measured. The dentin and an arbitrary standard of basic calcium phosphate, prepared from an aliquot of the active solution administered to the animals, were retained as standards. The ratio of the activity of the enamel to that of each of the standards was determined after each repurification of the enamel. In this way the natural decay of the radioactive phosphorus was prevented from influencing the comparative measurements of the activity of the specimens of enamel and dentin. A beta-ray Geiger-Mueller counter adapted for use with solids contained in uniform aluminum dishes 1 cm. in diameter was employed in the radioactivity determinations.

The ratio of the activity of one gram of enamel, after each repurification, to that of one gram of dentin and the ratio of the activity of the same enamel specimens to that of the arbitrary standard are shown in table 1. The data with reference to the ratio of the activity of the dentin to the calcium phosphate standard are included as a further indication of the precision with which the activity measurements were made over a period of five days. While no significant change in the activity of the enamel resulted from repurification from liquid of density 2.78 there was an apparent 6.9 per cent maximum decrease in the activity of the enamel after repurification from pure bromoform (density

2.85). Since the errors of four separate activity measurements are involved when any two ratios are compared, the significance of this apparent decrease is uncertain. Even if the decrease in activity is considered to be real, it can be shown by calculation that the removal of only 5 mgm. of dentin from each gram of the initial enamel specimen would account for the changed activity. At all events this result differs from that of Sognnaes and Volker (7) who found the most dense fractions of enamel to possess the highest activity. The activity measurements mentioned above were carried out on unaltered enamel and dentin and are useful only for purposes of comparison. Since the mineral content of enamel is considerably higher than that of dentin, the internal absorption of beta-rays by enamel is greater than that of dentin. Therefore, the true relative amounts of radiophosphorus acquired by enamel and dentin can be estimated only after the phosphorus contained in each is converted to the same compound. The purified specimens were ashed, dissolved in acid and precipitated as basic calcium phosphate. The ratio of the activity of one gram of enamel to that of one gram of dentin calculated from the activity measurements of the calcium phosphate precipitates was 0.0731. The phosphorus content of enamel exceeds

TABLE 1

Relation of radiophosphorus content of enamel to density

DENSITY OF ENAMEL*	RATIO OF ACTIVITY OF ENAMEL TO DENTIN	RATIO OF ACTIVITY OF ENAMEL TO ARBITRARY STANDARD	RATIO OF ACTIVITY OF DENTIN TO ARBITRARY STANDARD
>2.70	0.0775	0.997	12.87
>2.78	0.0774	1.026	13.26
>2.85	0.0721	0.957	13.27

* Density of liquid used in repurification of enamel. Density of dentin lay between 2.42 and 2.08.

that of the dentin (10). The rates of exchange of phosphorus by various tissues can be compared if the activity measurements are related to the phosphorus content of the several tissues. The ratio of the specific activities[2] of enamel to dentin was found, in this experiment, to be 0.0533. This figure indicates that the phosphorus of the enamel was exchanged at a rate which was approximately 5 per cent of that of dentin. Various parts of a femur of one animal were ashed and activity measurements were made on the calcium phosphate precipitated from the solutions of the ash. The following results, as percentage of the total quantity of radiophosphorus administered to the animals, were found for one gram weights of ash: enamel, 0.000546; dentin, 0.00878; femur epiphysis, 0.0611; femur diaphysis, 0.0133; and femur marrow, 0.310.

Sognnaes and Volker (7), as stated above, presented evidence that the largest fraction of the labeled phosphorus which enters the surface layer of enamel is derived from the saliva. An estimate of the quantity of active phosphorus acquired by enamel from saliva requires a knowledge of the active phosphorus

[2] Specific activity = per cent of administered radioactive phosphorus found per milligram of tissue phosphorus.

content of that fluid. If the plausible assumption is made that the inorganic phosphorus of plasma and saliva were, for most of the 5 days, in exchange equilibrium, it is possible to calculate the radioactivity of saliva from the specific activity measurements of plasma. The specific activities of the acid-soluble phosphorus of the plasma of three of the cats[3] were as follows: 0.00634, 0.00626 and 0.00555. From the average value 0.00605, and assuming 15.3 mgm. phosphorus per 100 cc. of saliva (11), it was calculated that the cats' saliva had, on an average, 65 counts of radioactive phosphorus per cubic centimeter as of the day on which the activity of their enamel was counted. From the results of *in vitro* experiments mentioned below, in which teeth were bathed in saliva to which labeled phosphorus had been added, it can be estimated that at least 5 per cent of the total radioactive phosphorus found in the cats' enamel was derived from their saliva. This figure represents the minimum fraction of the labeled phosphorus acquired by enamel from the saliva since the activity of the blood, and hence of the saliva, undoubtedly was higher for some hours after the subcutaneous administration of the labeled phosphorus than at the time the animals were sacrificed. However, it would appear from the observations of Hevesy (12), with reference to the change of labeled phosphorus content of plasma following the subcutaneous injection of radioactive phosphorus, that the active phosphorus content of the blood of the cats used in this experiment was never over three times the quantity calculated.

Three groups of human molar teeth, after their roots had been covered with collodion, were transferred to 15 cc. of saliva containing radioactive phosphorus. The solution was stirred for 24 hours at 38°. After removal from the saliva, the teeth were washed in running tap water and the roots were cut away and discarded. The uptakes of phosphorus by the enamel and dentin separated from these teeth were calculated from their respective activities and from the active phosphorus (954,000 counts per minute per 15 cc.) and total phosphorus (3.15 mgm. phosphorus per 15 cc.) contained in the saliva with which they were bathed. The enamel was found to have exchanged, per gram, 0.00478 to 0.00850 mgm. phosphorus. The dentin had exchanged, per gram, 0.000638 to 0.00541 mgm. phosphorus. The teeth used in this experiment showed no evidence of caries and did not possess noticeable cracks or flows. It is possible, however, that there were direct channels through the enamel from the occlusal surface. A variation of the size or capacity of the channels would account for the fact that there was no relation between the relative amount of phosphorus exchanged by the enamel and dentin of the teeth of the three groups. This experiment indicates what may occur in the way of exchange between saliva and dentin in the case of teeth, which are not entirely normal, but which probably represent the average condition of continuity of the enamel of many molar teeth.

In a similar *in vitro* experiment single-cusp human teeth were used. The incisal edge and any regions of the crown which, under magnification, remotely resembled a crack were covered with collodion. The teeth were left in contact with 3 cc. of a solution containing 150,000 counts of labeled phosphorus and 5.74 mgm. total

[3] The plasma of one animal was lost in centrifugation.

phosphorus for five days at a temperature of 38°. Tooth A was entirely covered with collodion and was used to determine the amount of phosphate which could traverse the collodion applied to the other teeth. It can be seen in figure 1 that this material is not absolutely impermeable to phosphate; nevertheless, the amount of labeled phosphorus acquired by this tooth was small. The bars labeled B represent the results in terms of uptake of phosphorus obtained on the analysis of the enamel and dentin of a tooth entirely covered with collodion except for an area of about 15 sq. mm. on the labial surface of the crown. It can be seen that considerably more radiophosphorus entered the enamel than was the case with tooth A. Since a significant exchange of phosphorus also occurred in the dentin, it must be concluded that this exchange occurred with the outer fluid and through the enamel. The bars marked C show the phosphorus ex-

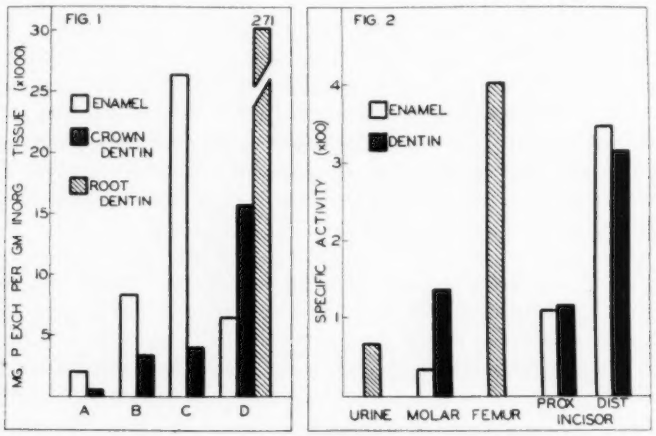

Fig. 1. In vitro uptake of phosphorus by enamel and dentin of human teeth.

Fig. 2. Specific activities of urine on the 103rd day and of calcified tissues on the 116th day following radiophosphorus administration to rats.

change by the enamel and dentin in the case of a tooth with a larger surface of the crown exposed to the solution. Here again the dentin acquired a small but measurable quantity of phosphorus through the enamel.

The crown of tooth D was covered with collodion *but the root, except for the apical foramen, was left bare.* It will be noted that the phosphorus exchange in the direction of greatest to least was root dentin, then crown dentin, and finally the enamel. The situation with regard to this tooth resembles that which would occur under *in vivo* conditions if exchange from the saliva were prevented. There can be little doubt that at least half of the labeled phosphorus which entered the enamel of tooth D migrated from the root dentin through the crown dentin.

Eight mature albino rats were sacrificed 116 days after having received by subcutaneous injection, a solution of sodium phosphate containing 23.5×10^6

counts of radiophosphorus and 11.2 mgm. total phosphorus. The solution was distributed among the animals in proportion to the $\frac{2}{3}$ power of their weight. The average specific activities of the femurs of the enamel and dentin of the molar teeth and of these fractions of the proximal and distal halves of the incisor teeth are compared in figure 2 with the specific activity of the urine on the 103rd day. Hevesy (12) has shown that the value of the specific activity of the urine, several days after administration of labeled phosphorus, closely approximates that of the plasma inorganic phosphate. In this experiment eight determinations of the urine specific activity were made at regular intervals, beginning on the 22nd day following injection of the radioactive phosphorus. The average results were found to decrease slowly and steadily from a value 8.6 times that finally recorded on the 103rd day. After this time the activity of the urine became too weak to determine with accuracy. In the 13 days intervening between the last measurements of urine activities and the sacrifice of the animals, the specific activity of the urine must have decreased. Therefore, the value for specific activity of urine indicated in figure 2 is clearly higher than that of this fluid, or of the plasma inorganic phosphate, at the time the animals were killed. The active phosphorus content of the blood collected on the 116th day of the experiment was too low for accurate measurement.

The activity of the molar enamel was slight but definitely significant. Even if the specific activity of the plasma inorganic phosphate had decreased, by the 116th day, to a value equal to that of the molar enamel, it would not be possible to conclude that all of the phosphorus of the molar enamel had been renewed in 116 days since a part of the labeled phosphorus had been incorporated in this enamel during the period when the plasma inorganic phosphate had a much higher activity than during the last days the animals lived. The specific activities of the tissues other than molar enamel, being higher than that of the plasma inorganic phosphate several days before the end of the experiment, indicates that the labeled phosphorus after entering these tissues left them more slowly than from the plasma. The same facts also show that, at the end of the experimental period, the radioactive isotope of phosphorus was leaving the same tissues at a more rapid rate than that at which they were acquiring this isotope. It will be noted that the enamel and dentin of the distal halves of the incisor teeth had considerably higher specific activities than those of the proximal halves of these teeth. This result is to be expected since the incisor is a continuously erupting tooth and the distal portion was formed about 25 days before the proximal portion, at a time when the blood had a considerably higher activity. The fact that the distal incisal enamel had a higher activity than the dentin is further evidence that enamel exchanges its phosphorus at a slower rate than does dentin since at the time of formation both the dentin and enamel must have had the same specific activity.

SUMMARY

Following the parenteral administration of radioactive phosphorus the uptake, 5 days after the administration, of the isotope has been determined in the enamel,

dentin, and bone of cats. In a similar experiment with rats the uptakes of the labeled isotope were determined after 116 days. The existence of mechanisms for transport of phosphate from saliva to dentin *via* the enamel and from the blood to the enamel by way of the dentin has been established. The radiophosphorus acquired by enamel of fully formed teeth is derived in part from the saliva but apparently in largest amount from the blood. The phosphorus of the enamel of molar teeth of rats is not completely renewed by exchange in 116 days.

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BONE AND TISSUE PHOSPHATASE IN EXPERIMENTAL SCURVY AND STUDIES ON THE SOURCE OF SERUM PHOSPHATASE

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The source of the serum phosphatase[1] has been the object of considerable investigation. Kay (1) considers the phosphatases of serum and of the various tissues (bone, kidney and intestine) to be identical and that the phosphatase activity of the serum is due to "leakage" from tissues of higher content. A. Bodansky (2) suggests a non-osseous source for serum phosphatase since he has observed elevated serum phosphatase levels after carbohydrate ingestion in young fasting dogs. He and other investigators (3) suggest the liver as a source of serum phosphatase in view of the elevated serum phosphatase values obtained in jaundice. Armstrong and Banting (4) showed that extirpation of various organs, including the liver, does not lower the serum phosphatase activity but may actually increase it. On this basis they consider the bone as the sole source of serum phosphatase. The observation that bone diseases of certain types result in highly elevated serum phosphatase activity has led certain investigators (5) to believe that bone is a major source of the serum enzyme. A. Bodansky (2) states that the serum phosphatase increase may be considered in bone disease to be of osseous origin, in liver disease to be of hepatogenous origin, and after the administration of carbohydrates probably to be of mixed origin. He suggests that the normal serum phosphatase is of diverse origin.

In previous investigations (6, 7) it has been shown that there is a distinct lowering of the serum phosphatase activity in both infantile and experimental scurvy and that the subsequent administration of ascorbic acid results in an increase of the serum phosphatase activity. Because it might be expected that there would be a concomitant decrease in the phosphatase activity of the source of the serum enzyme, a study of the changes in the concentration of phosphatase in the tissues of scorbutic animals might throw considerable light on the source of phosphatase normally present in serum.

In the present investigation a study has been made of the phosphatase activity of the various tissues (including sera) from normal, scorbutic, and ascorbic acid-treated scorbutic animals with a view to determining the source of serum phosphatase.

[1] Phosphatase as used in the present communication refers to the enzyme with an optimum activity between pH 8.6–9.0.

EXPERIMENTAL. Guinea pigs of the desired age were separated into groups of 2 or 3 and housed in clean wire cages. The animals were fed either a scurvy-producing diet, consisting of equal parts of skimmed milk (heated at 90° for 12 hours), rolled oats and bran and supplemented by 1 cc. cod liver oil every 4 to 5 days, or a diet consisting of Purina soy bean meal chow and fresh vegetables. When ascorbic acid was to be given it was dissolved in water immediately before use and fed by dropper. Animals fed the scorbutogenic diet invariably showed clinical evidence of scurvy in 18 to 24 days. At autopsy, extensive hemorrhages and fragility of the bones were always observed. Histological[2] and x-ray confirmation of scurvy were obtained. When supplemented with adequate amounts of ascorbic acid the diet was adequate for normal growth of the animals, as was evident from the normal increase in weight of the animals and absence of all symptomatology.

Serum phosphatase determination. Blood for phosphatase estimations was obtained by cardiac puncture under light ether anesthesia. A modification of the Bodansky method (6) using 0.5 cc. of fresh serum incubated with sodium β-glycerophosphate (Eastman Kodak) in veronal buffer at pH 8.6 in the presence of 0.009 *M* Mg (final conc.) for 60 minutes at 37°C. Acid-soluble inorganic phosphate was determined by the method of Fiske and Subbarow (8) using a photoelectric colorimeter. The activity is expressed in units, each equivalent to 1 mgm. of inorganic phosphorus liberated per 100 cc. of serum under the conditions of the experiment.

Tissue phosphatase estimation. The following tissues were freshly excised and so selected that comparable sections were always taken: both *adrenals;* the proximal half of the *tibia;* half a *kidney* (cut in sagittal sections); a section of the *intestine* 4–5 inches long taken about 2 inches from the *pylorus;* one-quarter inch on each side of and including the *costochondral junction* of the 4th to 7th ribs; a section of *liver;* the lower *incisors*, including the jaw. The tissues were ground in a mortar with 10 cc. of chloroform-water and allowed to stand in the refrigerator for 24 hours with periodic shaking. The estimation of phosphatase activity was carried out as described for serum phosphatase. The results are expressed both as units per 100 cc. of extract and as units per 100 mgm. dry weight of tissue.

In table 1 the data for the analyses of the skeletal tissues of a number of animals are presented. The data for the other tissues analyzed are not presented because they confirm the observations of Harrer and King (9), that there are no significant differences in the phosphatase activity of intestinal mucosa, kidney and liver of normal and scorbutic guinea pigs. In addition to these tissues, no significant alterations in the activity of the adrenals of normal and scorbutic animals were found. It can be seen from table 1 that the changes are most marked in the case of bone (tibia) phosphatase. The results expressed on a dry weight basis are not sufficiently descriptive because the bulk of the weight of the bone represents inactive tissue. There are also variations in size of bone as a result of

[2] Histological examinations were carried out through the courtesy of Dr. Sidney Farber and Dr. Nathan Rudo, to whom we express our sincere thanks.

differences in age of the animals. In addition, the results expressed on a dry weight basis do not adequately take into account the decreased weight of bone due to rarefaction in scurvy. A comparison of the average weights of comparable bone sections from normal animals (212 mgm.) and scorbutic animals

TABLE 1

Phosphatase activity of blood, bone (tibia), rib and tooth of normal, scorbutic and ascorbic acid-treated guinea pigs expressed in units (milligram of acid soluble inorganic phosphate liberated from sodium-β-glycerophosphate at pH 8.6 in presence of 0.009 M Mg^{++} in 1 hour at 37° C.) per 100 ml. of extract and per 100 mgm. dry weight of tissue

ANIMAL NUMBER	AGE	PHOSPHATASE ACTIVITY							REMARKS
		Blood, (units) 100 ml. serum	Bone		Rib		Tooth		
			Units per 100 cc. extract	Units per 100 mgm. tissue	Units per 100 cc. extract	Units per 100 mgm. tissue	Units per 100 cc. extract	Units per 100 mgm. tissue	
			Normal animals						
	weeks								
1	6	22.5	87.4	3.9	33.2	2.6	66.7	2.1	Diet; Purina chow and fresh vegetables
2	6½	20.7	110.0	7.9	42.4	5.0	80.0	2.2	
3	6	14.7	83.0	4.3	31.0	3.2	48.9	1.9	
4	6	23.0	91.0	4.4	38.0	4.8	66.7	2.2	
5	11	5.5	63.6	4.4			38.0	1.1	
6	11	8.8	68.1	2.6	15.0	1.3	60.0	1.3	On vegetable diet supplemented with 6 mgm. ascorbic acid daily for 5 weeks
			Animals on scorbutogenic diet supplemented with 1 mgm. ascorbic acid daily						
7	10	24.7	69.8	3.3	25.6	2.4	36.6	1.0	On diet 4 weeks
8	11	10.8	81.5	3.9	17.6	2.0	26.8	0.5	On diet 5 weeks
9	11	4.9	41.4	2.1	12.0	1.4	24.5	0.7	On diet 5 weeks
10	10	3.2	21.1	1.0	5.9	0.6	37.8	1.0	On scorbutogenic diet supplemented with 1 mgm. ascorbic acid daily for 5 weeks. Scorbutic.
			Animals on scorbutogenic diet supplemented with 6 mgm. ascorbic acid daily						
11	11	9.3	64.3	2.8	13.5	1.9	24.9	0.7	On diet 5 weeks
12	11	12.5	58.2	2.2	17.1	2.1	27.8	0.7	On diet 5 weeks
13	11	9.0	54.0	1.9	21.1	2.2	24.1	0.7	On diet 5 weeks
			Animals on unsupplemented scorbutogenic diet (scorbutic animals)						
14	8	4.5	37.8	3.3	24.7	3.7	47.1	1.8	On diet 15 days
15	9	5.7	26.8	1.7	14.0	1.6	29.2	0.7	On diet 3 weeks
16	9	3.5	20.9		8.0	1.6			On diet 3 weeks
17	9	2.6	24.5	1.7	12.2	1.4	28.1	1.0	On diet 3 weeks
18	9	2.9	17.5	1.3	11.3	1.4	38.3	1.2	On diet 3 weeks
19	9	1.8	11.4	1.1	6.4	1.1	40.7	1.2	On diet 3 weeks
20	10	2.5	17.6	1.2	10.6	1.4	24.2	0.7	On diet 4 weeks
21	11	3.0	12.5	1.2	9.7	1.1	17.7	0.5	On diet 5 weeks

TABLE 1—*Concluded*

ANIMAL NUMBER	AGE	PHOSPHATASE ACTIVITY							REMARKS
			Bone		Rib		Tooth		
		Blood, (units) 100 ml. serum	Units per 100 cc. extract	Units per 100 mgm. tissue	Units per 100 cc. extract	Units per 100 mgm. tissue	Units per 100 cc. extract	Units per 100 mgm. tissue	
			Scorbutic animals treated with ascorbic acid						
22	*weeks* 10	14.2	74.0	3.1	9.0	0.9	23.6	0.7	Scorbutic, treated with 0.5 mgm. ascorbic acid daily; total given, 3 mgm.
23	12	9.6	102.0	3.7	33.7	3.2	50.4	1.2	Same as 22 except that total given was 5 mgm.
24	11	10.4	57.2	3.8	11.9		23.6	0.7	Treated with 1.0 mgm. ascorbic acid daily; total, 6 mgm.
25	13	9.5	195.0	8.0	102.4	11.9	77.4	2.0	Treated with 3 mgm. ascorbic acid daily; total, 27 mgm., then fed scorbutogenic diet 13 days then treated again in same way
26	9	0.7	38.4	1.6	10.6	1.6	43.2		Scorbutic, treated with 6 mgm. ascorbic acid for 3 days, died of pneumonia
27	14	9.0	72.5	2.6	12.4	0.9	17.6	0.4	Treated with 6 mgm. daily, total 78 mgm.
28	10	8.0	62.7	3.0	41.4	4.7	55.1	1.8	Treated with 6 mgm. daily, total 24 mgm.
29	12	14.8	80.6	2.7	26.3	1.9	33.0	1.1	Treated with 6 mgm. daily, total 78 mgm.
30	12	10.6	71.5	3.1	16.6	1.4	16.5	0.4	Treated with 12 mgm. daily, total 96 mgm.

that received treatment (241 mgm.) with those from scorbutic animals (151 mgm.) indicates quite clearly that an analysis of activity on the basis of the activities of extracts, similarly prepared from comparable sections is more significant.

The decrease of phosphatase activity of scorbutic bone (tibia) is definite and parallels the decrease in the serum activity of scorbutic animals. With treatment, even for as short a time as six days and with doses as small as 0.5 mgm. ascorbic acid daily, there is an increase in the bone phosphatase. On the other hand, the administration of large doses of ascorbic acid to normal animals does not result in an increase in bone phosphatase significantly above normal. With a few scorbutic animals (animals 23 and 25) the administration of rather large doses of ascorbic acid resulted in elevated levels in the bone. The increase in bone phosphatase activity when ascorbic acid is administered to scorbutic animals is not proportional to the dose given, either to daily intake or to total ascorbic acid administered. This has been shown to be the case with serum (7).

In respect to the phosphatase activity of rib the differences are not so pronounced as in the tibia. There is, however, a definite decline in the rib phos-

phatase in scurvy and a somewhat slower recovery than in the tibia following treatment. In the case of the activity of tooth phosphatase, from the data available, no significant differences can be observed. It is possible that with the method employed small changes in phosphatase activity of the tooth might be obscured by the presence of a large amount of jaw contained in the tooth samples.

It should be pointed out that there is a decrease in the activity of phosphatase of bone, rib and teeth with increasing age of the animal. Robison (10) has demonstrated similar decreases in bone phosphatase with increasing age for a number of species. It is for this reason that the data for each animal are presented separately.

Osteoblasts as source of serum phosphatase. The decrease or increase in bone phosphatase activity and the concomitant fall or rise in the serum phosphatase in active scurvy or after vitamin C administration, whereas other tissue phosphatase variations do not appear to be significant, suggest rather strongly that the source of the serum phosphatase is largely, if not entirely, the bone.

It has been shown by Wolbach and Howe (11) that in scurvy the connective tissue cells of the marrow of scorbutic animals are migrated osteoblasts which have reverted to fibroblasts. In the scorbutic process, therefore, we may have a state of modified osteoblastic activity or a diversion of osteoblastic activity from bone formation. The phosphatase activity of bone appears to reflect the activity of the osteoblasts. In active scurvy where these cells are relatively inactive the concentration of the enzyme is markedly reduced and this is reflected in a low serum phosphatase. Upon treatment of the scorbutic animal with ascorbic acid a return of normal osteoblastic function ensues and there is a concomitant increase in the activity of the bone and serum phosphatase. Thus our findings are in agreement with the conclusion of Robison (10) that the bone phosphatase is of osteoblastic origin.

Nature of the serum phosphatase in scurvy. (*Bile salt differentiation.*) O. Bodansky (12), using 0.067 M taurocholate or glycocholate, inhibited bone and kidney phosphate activity about 50 per cent, while intestinal phosphatase activity was not inhibited. Unfortunately, he did not study guinea-pig preparations. He used "semipurified" preparations isolated from 2 to 5 day autolysates at room temperature rather than fresh tissue extracts. To the small amount of phosphatase that remains in the sera of scorbutic animals, bile salts were added in an attempt to determine whether this residual phosphatase activity might possibly be of intestinal origin. In addition, a series of experiments was carried out on freshly isolated guinea-pig bone, kidney, and intestinal extracts (24-hour extract with $CHCl_3$-water at 10°C.). Estimations were made, as described previously, except that where indicated the reaction mixture contained the indicated concentration of bile salts or sodium taurocholate.

An analysis of table 2 shows that when 0.072 M taurocholate is used with fresh extracts of guinea-pig intestine, there is definite inhibition of phosphatase activity but of a lesser degree than is observed for bone or kidney phosphatase. If the taurocholate concentration is lowered to 0.009 M intestinal phosphatase is not inhibited, whereas bone and kidney phosphatases do show definite inhibition. If autolysis is prolonged over a period of 5–6 days the taurocholate

inhibition of intestinal phosphatase is decreased and in some cases disappeared. Rabbit intestine phosphatase prepared by the method used in the present study showed no inhibition with 0.067 M or 0.072 M sodium taurocholate indicating a definite difference between guinea-pig intestinal phosphatase and those of the various species studied by O. Bodansky (12).

Serum phosphatase estimations of a number of normal and scorbutic animals were carried out in the absence of bile salts or sodium taurocholate and in the presence of 0.4 per cent bile salts or 0.009 or 0.072 M sodium taurocholate. The results shown in table 2 indicate no significant differences in the degree of inhibition of the sera of normal and scorbutic animals. While this does not indicate that the residual activity normally encountered in the sera of scorbutic

TABLE 2

*Inhibition of bone, kidney, intestine, and serum phosphatases by sodium taurocholate and bile salts**

TISSUE	TAUROCHOLATE			BILE SALT	
	Number of samples	0.009 M	0.072 M	Number of samples	0.4 per cent
		per cent inhibition	*per cent inhibition*		*per cent inhibition*
Bone	3	21	53	4	42
Kidney	3	21	54	4	41
Intestine	10	4	35	12	26
Blood†	2		53	9	43
(normal)	4	26			
Blood	3		53	9	42
(scorbutic)	5	25			

* Sodium taurocholate (Hoffmann-La Roche). Bile salt (Fairchild, mixture of sodium taurocholate and glycocholate).

† Average normal serum phosphatase, 11.6 units. Average scorbutic phosphatase, 3.5 units.

animals is of osseous origin it does indicate that it is not largely of intestinal origin. Inasmuch as the serum phosphatase level in scurvy has approached zero in a number of instances and in view of the fact that the bulk of the serum phosphatase has been identified as of osseous origin, it is not unlikely that all of the serum phosphatase under normal conditions originates in the bone.

SUMMARY AND CONCLUSIONS

The phosphatase activity of the tissues of a large group of normal and scorbutic guinea pigs was studied. No significant differences between the phosphatase activity of normal and scorbutic guinea-pig intestinal mucosa, kidney, adrenal or liver were observed.

The phosphatase activity of bone (tibia) from scorbutic animals is strikingly decreased. In rib the decrease is not so pronounced. With recovery from scurvy the bone phosphatase is restored to its normal activity. The fall in bone

phosphatase activity parallels the fall in serum phosphatase activity and with the administration of ascorbic acid there is a concomitant rise in the blood and bone phosphatase.

These findings together with the histological findings in scurvy suggest that osteoblasts are the source of the serum enzyme.

The inhibitory effect of bile salts suggests that the small amount of phosphatase activity remaining in the serum in severe scurvy is not of intestinal origin.

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BLOOD LEVEL OF MAGNESIUM ION IN RELATION TO LETHAL, ANESTHETIC, ANALGESIC AND ANTITETANIC EFFECTS

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The anesthetic effect of parenterally administered magnesium salts was demonstrated by Meltzer and Auer in 1905, and the serum concentrations necessary for this action were investigated by Neuwirth and Wallace in 1929. The effect of magnesium salts in inhibiting pain reactions elicited by intra-arterial injections of potassium chloride or acids was demonstrated by Moore (1934), and the use of magnesium sulfate to relax muscle spasm in tetanus, suggested by Meltzer and Auer, was reviewed by Moore and Singleton (1939).

The present study concerns 1, the fatal blood level of magnesium ion; 2, the respiratory nature of death caused by magnesium administration; 3, the antagonism of calcium and magnesium, and 4, the serum level of magnesium necessary to prevent pain reactions elicited by intra-arterial injections of potassium chloride. Furthermore, although only one case of tetanus was available for study, by administering to normal patients the dose of magnesium sulfate found effective in tetanus we have estimated 5, the antitetanic blood level of magnesium ion.

METHODS. Experiments were performed upon 70 cats and upon 6 dogs, anesthetized with a barbiturate (sodium amytal, nembutal) or with ether. Continuous intravenous injections were made using 2.16 per cent $MgCl_2 \cdot 6H_2O$, which is isotonic with blood. The injections were made from a burette into the brachial or the saphenous vein. The rate of injection approximated 1.0 cc. per minute in cats or 4.0 cc. per minute in dogs. Blood samples for analysis were taken from the inferior vena cava in the case of brachial injections, from the heart with saphenous injections. Magnesium determinations were made, at first on plasma and later on serum, by the method of Hirschfelder and Serles (1934), modified in that the color comparison was made using a series of standards in uniform test tubes. In experiments in which the serum calcium was also determined the Clark-Collip modification of the Kramer-Tisdall method (1925) was used.

In determining the serum level of magnesium required to prevent the pain reactions elicited by intra-arterial injections of potassium chloride, tests were made according to the method of Moore (1934). Three isotonic test solutions were used: 1, isotonic KCl (1.15 per cent), 2, a mixture of 7 parts isotonic KCl and 3 parts isotonic NaCl (0.8 per cent KCl), and 3, a mixture of 4 parts isotonic

KCl and 6 parts isotonic NaCl (0.46 per cent KCl). When enough magnesium chloride had been given to prevent pain reactions from the 0.46 per cent KCl mixture a blood sample was taken and the magnesium level determined. Tests were then continued with the 0.8 per cent KCl mixture until it failed to elicit a response when another blood sample was taken. Finally the magnesium chloride injection was continued until the reaction to pure isotonic KCl had disappeared.

RESULTS AND DISCUSSION. *The fatal blood level of magnesium ion and the respiratory nature of magnesium death.* Continued intravenous administration of magnesium chloride caused a gradual decrease in the respiratory rate followed by a characteristically abrupt cessation of respiration. Moreover, prior to death the heart beat was noticeably slowed. In 13 cats under nembutal or sodium amytal anesthesia the serum magnesium concentration found at death averaged 24.7 mgm. per 100 cc. (range—21.0 to 31.0 mgm.), while in 2 dogs under nembutal anesthesia the magnesium levels at death were 24.0 and 32.0 mgm. per 100 cc. When ether was used for anesthesia and the ether discontinued after sufficient magnesium chloride had been administered to keep the animal quiet, considerably higher fatal levels were obtained. Thus, in 7 cats which were given ether the serum magnesium concentration at death averaged 33.0 mgm. per 100 cc. The range (23.0 to 50.0 mgm.) was greater than with barbiturates, probably because of variation in the amount of ether used. Similarly, in 4 dogs under ether, death levels were 26.0, 30.0, 48.0 and 54.0 mgm. per 100 cc.

That sublethal doses of magnesium chloride are innocuous was demonstrated in 7 animals in which the experiment was performed aseptically and the magnesium injection discontinued before the stage of respiratory paralysis. Although the serum magnesium concentration had reached 20 to 27 mgm. per 100 cc. the animals recovered without apparent ill effect. In 4 unanesthetized animals 25 per cent magnesium sulfate was given subcutaneously (0.9 gram per kgm. body weight) and the animals survived, the blood concentrations ranging from 23 to 34.0 mgm. per 100 cc.

These experiments indicate that when magnesium is used in anesthetic doses the only serious danger lies in the possibility of respiratory death through slight overdose. This conclusion was verified by 5 experiments in which respiration was maintained artificially. The heart beat remained perceptible to palpation until a serum magnesium level averaging 60.3 mgm. per 100 cc. was reached (range—42.0 to 72.0 mgm.).

Antagonism of calcium and magnesium. Meltzer and Auer recommended the use of calcium salts to counteract the respiratory depression resulting from overdoses of magnesium. The value of calcium ion in this respect was demonstrated by us in 6 experiments in which a double-isotonic magnesium-calcium mixture, containing isosmotic concentrations of the two chlorides, was injected. In these animals the magnesium level at death averaged 65.8 mgm. per 100 cc. (range—45 to 94 mgm.) while the serum calcium averaged 36.9 mgm. per 100 cc. (range—28 to 50.5 mgm.). Furthermore, when death occurred it was characterized by a sudden weakness and irregularity of the heart beat, with respiratory move-

ments persisting. On opening the chest it was noted that although the heart was moderately contracted it did not appear to be in calcium rigor; we presume that the high magnesium concentration accounted for this.

Serum level of magnesium preventing pain reactions elicited by intra-arterial injections of potassium chloride. In these experiments some very interesting results were obtained. In the animals with preliminary ether anesthesia it was noted that the serum magnesium concentration required to abolish the pain reactions elicited by the isotonic mixture containing 0.46 per cent KCl was approximately that required to abolish the pain of skin incision. The concentration effective in preventing pain reactions from the mixture containing 0.8 per cent KCl was that which produced average surgical anesthesia permitting laparotomy and manipulation of the viscera. On the other hand, reactions to the injection of pure isotonic KCl persisted until the serum magnesium concentration approached lethal values. In 15 experiments with ether the magnesium concentration preventing stimulation by 0.46 per cent KCl or by skin incision averaged 15.1 mgm. per 100 cc. (range—11.7 to 18.0 mgm.). In the same experiments it was necessary to attain a magnesium concentration of 23.5 mgm. per 100 cc. (range—18.5 to 27.0) in order to prevent stimulation by 0.8 per cent KCl or by laparotomy. Most of the cats were dying from respiratory depression by magnesium at the time the reaction to pure isotonic KCl disappeared.

In animals under light nembutal anesthesia comparable analgesic effects were obtained with lower magnesium concentrations. The stage of surgical anesthesia was reached and the reactions to 0.8 per cent KCl disappeared when the serum magnesium reached 12 to 13 mgm. per 100 cc. Moreover, the response to isotonic KCl (1.15 per cent) was abolished at magnesium concentrations of 20 to 22 mgm. per 100 cc. It is apparent from these experiments that the analgesic actions of magnesium and nembutal are additive.

The antitetanic blood level of magnesium ion. On the basis of clinical studies, Moore and Singleton (1939) recommended the intramuscular injection of 25 per cent magnesium sulfate combined with luminal for the control of muscle spasm in tetanus. In their cases an injection of 4 to 10 cc., depending upon the body weight, was given at four-hour intervals.

In ten convalescent hospital patients the normal serum magnesium concentration was 2.7 to 4.6 mgm. per 100 cc. (average—3.8). In four adult patients a single injection of 10 cc. of 25 per cent magnesium sulfate was given and the serum concentration followed for some hours. The maximum elevation to 6.0–6.5 mgm. per 100 cc. was attained between one and two hours after injection; the concentration then diminished slowly until at end of 12 hours it approached the original level. In two patients the same amount of magnesium sulfate was injected subcutaneously in isotonic solution. Elevations of serum magnesium to 6.5 and to 5.5 mgm. per 100 cc. were obtained after 2 and 3 hours respectively.

The effect of repeated injections is illustrated by determinations in the one case of tetanus which was available. The patient, a boy of twelve years, was given 4 cc. injections of 25 per cent magnesium sulfate at two-hour intervals for

48 hours. The highest concentration obtained was 7.0 mgm. per 100 cc. The tetanus remained uncontrolled. Later, the same dosage at three-hour intervals, combined with luminal, relieved muscle spasm. After 72 hours of this treatment the serum magnesium concentration was 6.5 mgm. per 100 cc. These observations indicate that with repeated doses no dangerous cumulative effect occurs.

SUMMARY

Continued intravenous administration of magnesium salts to cats and dogs produces anesthesia. In cats with preliminary etherization death from sudden respiratory collapse occurs at serum magnesium concentrations averaging 33.0 mgm. per 100 cc. In cats in which magnesium administration is combined with light sodium amytal or nembutal anesthesia the lethal serum concentration is distinctly lower, averaging 24.7 mgm. per 100 cc. Essentially similar results are obtained in dogs.

Higher serum magnesium concentrations are tolerated if respiration is maintained artificially or if calcium salts are administered. If magnesium administration is terminated before death, the animal survives, apparently uninjured.

With preliminary ether anesthesia, serum magnesium concentrations of about 15 mgm. per 100 cc. prevent pain reactions elicited by skin incision or by intra-arterial injection of an isotonic KCl-NaCl mixture containing 0.46 per cent KCl. Serum concentrations of about 23 mgm. per 100 cc. provide satisfactory surgical anesthesia and prevent pain reactions from intra-arterial injection of a mixture containing 0.8 per cent KCl. Near-fatal concentrations are required to prevent reactions to the strong stimulation of pure isotonic KCl (1.15 per cent). In contrast, with combined barbiturate and magnesium anesthesia, the magnesium concentrations required for these effects are significantly lower.

From studies in one case of tetanus and in normal patients given magnesium sulfate in doses used in tetanus, it appears that dangerously high serum concentrations of magnesium are not required for effective antitetanic action.

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CALCIUM IN GASTRIC MUCUS AND REGULATION OF GASTRIC ACIDITY

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In a recent investigation (1) it was found that the calcium concentration of gastric secretions, as a rule, varied inversely with the acidity of the secretions. This relationship was seen most convincingly in the differences between secretions of the vagal and histamine type produced alternately in an animal.[1] The source of the calcium and its relationship to acidity changes interested us. Experiments in which changes in acid placed in the stomach were determined before and after the production of mucus were supplemented by *in vitro* experiments in which mucus was exposed to acid in a flask. Evidence was thus obtained that mucus is a source of calcium and an important factor in acidity reduction. This provided an explanation for calcium and acidity variations in gastric secretions.

EXPERIMENTAL PROCEDURE. Cats fasted 24 hours were given chloralose-urethane intravenously under ether. The trachea was cannulated, the duodenum tied off, and the esophagus blocked by means of a cork drawn up through the cardia and held in place by stitching the cord to which it was attached through the wall of the esophagus in the neck. The stomach was fistulated, a wide glass tube being used. In an hour's time the vagi were cut, the stomach washed with warm normal saline and the experiment proper begun. In the control period three samples (either 7 cc. or 15 cc.) of isotonic HCl of known strength (130 or 160 m.eq./l.) were placed successively at 15-minute intervals in the stomach. The acid was removed completely at the end of each interval by draining it into a measuring tube. It was filtered and analysed for calcium, and free and total acidities as in the previous work (1). An hour was then allowed to elapse before the introduction of a mucus-producing agent (either 1 per cent acetic acid for ½ hour or 80 to 95 per cent alcohol for 5 min.). One hour after the removal of this agent three samples of HCl were successively introduced as in the control period. This procedure permitted a comparison

[1] Since this paper was written work by Gray and Bucher (2) has been published, which has confirmed the inverse relationship between calcium concentration and acidity. These workers conclude from their statistical calculations that the calcium is derived from the non-parietal fraction of gastric secretions, which agrees with our previous findings (1) that at the height of secretion of the histamine type the calcium concentration is either very low or it may disappear entirely, while secretion produced at the same rate through vagal faradization in the same animal contains relatively large amounts of calcium.

to be made of the acidity reduction in the stomach before and after the production of considerable quantities of gastric mucus.

In some of these experiments the mucus was collected from the stomach after the last sample of acid had been removed, measured in a 15 cc. graduated centrifuge tube, a known portion ashed for calcium and the remainder exposed to a sample of control HCl in a flask. (The volume of HCl used was calculated to give approximately the ratio of acid to mucus which had existed in the stomach.) The flask was left in some cases for 15 minutes at 37°C., and in a few other cases overnight in the refrigerator. Care was taken to avoid mixing in order to prevent the mucus mass from being broken up, and, as a rule, the mucus and acid recovered finally were within a few tenths of a cubic centimeter of their original volumes. On the recovered acid, calcium and acidity were determined, while on the recovered mucus calcium was estimated. In another series mucus which had *not* been exposed to HCl introduced into the stomach was collected and exposed to HCl in a flask, but in these cases three samples of acid were added successively at 15-minute intervals as in the *in vivo* experiments, each being recovered and finally with the recovered mucus analysed separately. In this series the volume of mucus was smaller in proportion to the volume of HCl used, than under the *in vivo* conditions, since without doubt a certain unknown amount of mucus would have remained on the surface of the stomach and escaped being collected.

Mucus was examined microscopically in the fresh state with dim illumination.

Results. The acidity reduction and calcium uptake in acid placed in the stomach before and after the production of mucus by alcohol or by acetic acid are shown in table 1. For the two strengths of acid used the differences in results were insignificant. The volume of HCl introduced, however, influenced the relative changes, in agreement with Toerell's findings (3); so it was important to keep this factor constant.

In the control and mucus groups the acidity reduction and calcium uptake were always greatest in the first 15-minute period and progressively fell off. A great increase over the control values was found in the extent of acidity reduction and calcium uptake by HCl in stomachs containing mucus.

That these changes were due to mucus and not to other factors at work in the stomach was shown by the results of *in vitro* experiments given in tables 2 and 3. The first series (table 2) showed that mucus was a source of calcium and an important factor in acidity reduction. The results were of more qualitative importance than quantitative, since the volumes of mucus available in individual experiments were too small for quantitative accuracy, and the mucus in these cases had been exposed to HCl introduced into the stomach before it was collected for the *in vitro* experiments, which made the original calcium concentration low. In the second series (table 3), mucus which had *not* been exposed to HCl in the stomach was exposed to HCl in flasks under conditions analogous to those of the *in vivo* experiments. The results were similar to those found when the procedure was carried out in the stomach (for comparison see table 4), taking into consideration the fact that neutralization by a cer-

tain amount of mucus undoubtedly remaining in the folds of the stomach would be effective *in vivo* but left out of the reckoning *in vitro*. The calcium concentration of the mucus originally was calculated from the calcium recovered in the acid samples + that recovered finally from the mucus.

TABLE 1

Summary of results for acidity reduction and calcium uptake in isotonic HCl solutions introduced into the stomach

GROUP AND PERIOD	ACIDITY REDUCTION		NUMBER OF CATS	CALCIUM UPTAKE		NUMBER OF CATS
	Average	Range		Average	Range	
	m.eq./l.	*m.eq./l*		*mgm./100 cc.*	*mgm./100 cc.*	
Control: with 7 cc. HCl (130 m.eq./l.):						
Period 1	35	29– 40	7	0.42	0–0.72	5
Period 2	16	12– 22	7	0.21	0–0.58	5
Period 3	13	11– 16	7	0.09	0–0.48	5
Control: with 15 cc. HCl (130 m.eq./l.):						
Period 1	19	17– 22	3	0	0	3
Period 2	9	8– 10	3	0	0	3
Period 3	7	6– 7	3	0	0	3
Control: with 7 cc. HCl (160 m.eq./l.):						
Period 1	39	27– 44	8	0.08	0–0.35	8
Period 2	23	17– 32	8	0.11	0–0.50	8
Period 3	19	14– 28	8	0.13	0–0.35	8
Mucus after alcohol: with 7 cc. HCl (160 m.eq./l.):						
Period 1	95	79–119	12	2.38	1.00–4.75	12
Period 2	68	53– 89	12	2.06	0.90–3.50	12
Period 3	56	44– 84	12	1.54	0.90–2.40	12
Mucus after acetic acid: with 7 cc. HCl (130 m.eq./l.):						
Period 1	61	45– 79	7	2.29	1.77–3.20	6
Period 2	38	24– 57	7	1.33	0.85–1.90	6
Period 3	25	15– 43	7	0.77	0.30–1.75	6

The variations from one experiment to another would probably have been less had the size of the stomach, which governed the amount of mucus produced, been under control. The volumes of mucus produced varied from 3 cc. to 7 cc. Macroscopically, this mucus which was alkaline to litmus, could be described as opaque, greyish-white and of a jelly-like consistency, and indistinguishable from mucus frequently seen in gastric secretions of the dog. Microscopic examination of a fresh unstained sample with dim illumination showed

that it contained, suspended in transparent material (mucus), which included globules of various sizes, very great numbers of cells which by their appearance and from the fact that they took up mucicarmine were identified as mucus-secreting cells. This opinion was supported by the report of Dr. H. Selye, who kindly examined the mucus with nuclear stains. These cells, however, were identified more clearly in fresh mucus. Vagal secretion, which is not as liquid as histamine secretion but usually viscous to varying degrees although seldom to the degree which could be described as jelly-like, was transparent and showed no cellular elements when examined microscopically. The same cell was found in mucus secreted spontaneously in the dog and cat.

TABLE 2

Loss of calcium from gastric mucus after exposure in vitro for 15 minutes to HCl with original acidity of 130 m.eq./l.

CALCIUM IN MUCUS*			CALCIUM LOSS FROM MUCUS	CALCIUM GAIN BY ACID	LOSS OF ACIDITY
Before exposure to acid		After exposure to acid			
mgm./100 cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*m.eq./l.*
3.50	0.115	0.018	0.097	0.101	41
4.69	0.082	0.000	0.082	0.067	23
4.69	0.090	0.034	0.056	0.060	19
2.60	0.052	0.000(?)	0.052	0.024	24
2.20	0.051	0.013	0.038	0.042	34
4.47	0.156	0.046	0.110	0.093	36
1.16	0.035	0.022	0.013	0.008	35
1.73	0.052	0.030	0.022	0.012	12
2.40	0.084	0.040	0.044	0.053	18
3.11(?)	0.096	0.048	0.048	0.134(?)	38
1.80	0.036	0.000	0.036	0.000(?)	10
1.55	0.026	0.000	0.026	0.028	29
2.40	0.034	0.000	0.034	0.012	15
5.81	0.154	0.062	0.092	0.066	41

* The mucus in all cases had been exposed to HCl in the stomach before it was removed for *in vitro* experiments. The original calcium concentration averaged 3.01 mgm./100 cc. with variations from 1.16 to 5.81 mgm./100 cc.

Although I did not succeed in obtaining an alkaline vagal secretion at will in cats, as Vineberg (4) has reported for dogs, by using a weak induced shock, nevertheless in one experiment on a dog increase in strength of stimulus following a period of weak stimulation produced an alkaline secretion of 8.4 cc. in 15 minutes. This had a calcium concentration of 8.3 mgm./100 cc. and a pepsin value of only 28 Mett units.

Discussion. It appeared improbable that the calcium source was either the parietal or the peptic cell since we had found that the calcium disappeared from gastric juice at the height of the secretion under conditions when chiefly the parietal cells (histamine) or the peptic and parietal cells (sham-feeding—unpublished data), were functioning. In addition, Stavraky (10) had found a

TABLE 3

In vitro experiments: Acidity reduction and calcium uptake in HCl with original acidity of 130 m.eq./l. after exposure of fresh 7-cc. samples to mucus in 3 successive 15-minute periods in a flask

CAT	PERIOD	ACIDITY REDUCTION	CALCIUM UPTAKE	CALCIUM IN MUCUS ORIGINALLY*	VOLUMES OF HCl AND MUCUS USED	MUCUS-PRODUCING AGENT
		m.eq./l.	*mgm./100 cc.*	*mgm./100 cc.*		
1	1	40	1.80		2.8 cc. mucus + 7.0 cc. HCl	1% acetic acid
	2	17	1.05			
	3	9	0.35			
2	1	40	0.90	6.00	3.0 cc. mucus + 7.0 cc. HCl	70% alcohol
	2	15	0.65			
	3	6	0.45			
3	1	46	1.85	10.10	3.6 cc. mucus + 7.2 cc. HCl	1% acetic acid
	2	24	1.15			
	3	6	0.70			
4	1	56	1.60	10.48	5.2 cc. mucus + 7.0 cc. HCl	1% acetic acid
	2	29	1.30			
	3	11	0.80			
5	1	37	1.40	8.75	3.3 cc. mucus + 7.0 cc. HCl	1% acetic acid
	2	20	1.15			
	3	8	0.80			
6	1	53	1.65	7.37	4.5 cc. mucus + 7.0 cc. HCl	1% acetic acid
	2	28	1.30			
	3	14	0.60			

* The Ca concentration of the mucus before exposure to HCl was calculated from the Ca recovered in the acid samples + that recovered finally from the mucus. Average Ca = 8.54 mgm./100 cc. with variations from 6.00 to 10.48. (N.B. The mucus in these cases had *not* been exposed to HCl before being used for the *in vitro* experiments.)

TABLE 4

Comparative results for acidity reduction and calcium uptake in vivo and in vitro

	ACIDITY REDUCTION		NUMBER OF CATS	CALCIUM UPTAKE		NUMBER OF CATS
	Average	Range		Average	Range	
In vivo experiments (HCl + mucus *in stomach*)						
	m.eq./l.	*m.eq./l.*		*mgm./100 cc.*	*mgm./100 cc.*	
Period 1	61	45–79	7	2.29	1.77–3.20	6
Period 2	38	24–57	7	1.33	0.85–1.90	6
Period 3	25	15–43	7	0.77	0.30–1.75	6
In vitro experiments (HCl + mucus *in flask*)						
Period 1	46	40–56	5	1.66	1.40–1.85	5
Period 2	23.6	17–29	5	1.19	1.05–1.30	5
Period 3	9.6	6–14	5	0.65	0.35–0.80	5

The mucus-producing agent was 1 per cent acetic acid; 7-cc. volumes of HCl, the original strength of which was 130 m.eq./l., were used.

high calcium with no pepsin in an alkaline mucoid secretion obtained from a dog after the intra-arterial injection of acetylcholine; and also we had found a high calcium with low pepsin in an alkaline secretion produced during vagal faradization in the dog. Thus calcium appeared in the gastric juice whenever the mucous or mucoid cells were secreting. The finding that mucus from the surface epithelium is a source of calcium and the parallel found between acidity loss from, and calcium uptake by, the acid seem to indicate that the appearance of calcium is an index of a reaction of acid with mucus in which the acidity loss is brought about. The loss which occurs in the control period before the production of mucus is probably due to small amounts of mucus on the surface of the stomach, which according to Babkin (5, 6) are being continuously produced. The small amounts of calcium sometimes found in the control samples lend support to this assumption since mucus is the source of the calcium. Very small amounts would be missed with the volumes used for analysis.

The calcium might have come from either the mucus secretion itself or from the mucus-secreting cell which I have found this type of mucus to contain in large numbers. From the relatively high concentration of calcium in vagal secretion which we have repeatedly found and since this secretion may be alkaline and viscous but devoid of cellular elements, we believe the source of the calcium is the mucus rather than the body of the mucus-secreting cell. Whether or not the mucus in alkaline vagal secretion is identical in composition with the mucus part of opaque "mucus" (mucus + secreting cell) is not known. Our figures for calcium concentration, of the same order in both, give us no basis for supposing that a difference exists. It may be that mucus from all sources in the mucosa is identical but under some conditions may contain the secreting cell which gives it an opaque and jelly-like appearance. If mucus proves to be the sole source of calcium, as our results suggest, and if it has a constant value in the product of these cells, a convenient method might be made available for estimating the output of mucus-secreting cells.

The appearance of calcium in gastric secretion when a certain group of cells of the gastric mucosa is functioning, is a striking example of the independent working of different epithelial secretory groups under control of different mechanisms, which was emphasized by Babkin in 1931 (5).

That much if not all of the acidity loss in these experiments was due to the mucus factor and in no way dependent on other factors in the stomach was shown by the experiments in the flask. It must be pointed out that under both *in vivo* and *in vitro* conditions, the contact with mucus must have been even less effective for reaction purposes than it would be when acid is secreted under physiological conditions. There can be little doubt that the acidity loss in gastric juice secreted in dog's *stomachs* and *pouches* in which excess mucus had been produced by 1 per cent acetic acid introduced into the *stomachs*, observed by Gorbunova *et al.* (7) and by Babkin *et al.* (8), was primarily if not entirely due to the presence of the excess mucus.

In Teorell's (3) theory for the regulation of acidity by the diffusion of H ions in exchange for Na ions, the region from and to which these ions migrate is not

established. They are depicted as entering and leaving the stomach. Though we cannot deny the possibility of the exchange of ions on which this theory is based, the fact that acidity loss in our experiments was found to be primarily if not entirely dependent on mucus suggests that the hypothetical migration of the ions may be into and out of the mucus. Our work gives direct support to the theory held by Babkin and by others that the epithelial-mucous border contains a mechanism which forms the first line of defence against changes in acidity. Mahlo and Mulli (9) in discussing this theory recently express the opinion that the mucus probably plays a major rôle in acidity regulation owing to its ability to adsorb (or to chemically bind) HCl, or to set it free when necessary, whichever is necessary to maintain the pH at the required level. The continuous production of small amounts of mucus which Babkin has stressed is of prime importance for this theory.

The lack of knowledge about the physical and chemical properties of gastric mucus has become increasingly apparent. The type of mucus produced by the agents we have used was that usually described as "visible" and was indistinguishable in appearance from mucus sometimes found in the fistulated stomachs or pouches of dogs and in vagal secretion. Our finding that this type contains large numbers of mucus-secreting cell throws new light on a number of controversial subjects. Since the decision seems justifiable that these cells secrete the alkaline mucus in which they are suspended, the ability of the mucosa to produce an alkaline secretion must be admitted. The opacity and jelly-like consistency of this visible type can be explained by the presence of the secreting cell in large numbers and these could also be responsible for the difference in appearance between the visible or opaque and the soluble or transparent type. The loss of these cells from the mucosa in large numbers under some conditions appears to be a normal phenomenon, since the cells have been found in mucus secreted spontaneously in the dog and cat, and 60 per cent alcohol, the weakest yet tried, in contact with the mucosa for 5 minutes produces as much of this mucus as the stronger concentrations. Interesting physiological problems are suggested by this and some of these are at present being investigated.

SUMMARY AND CONCLUSIONS

Results for acidity reduction in, and calcium uptake by, isotonic HCl of physiological strength placed in the cat's stomach before and after the production of gastric mucus, and similar changes found under *in vitro* conditions when mucus collected from the stomach was exposed to HCl in flasks, are described. These, combined with some further observations added to our original findings on the composition of gastric secretions of the vagal type, have led to the following conclusions.

1. Mucus from surface epithelial cells and from the cells producing it in the vagal type of secretion is an immediate source of calcium in gastric secretions. Actively functioning peptic or parietal cells do not appear to be necessary for its production.

2. The theory that mucus is an important factor for the reduction of gastric acidity is supported by direct evidence and the migration of H ions out of the stomach is not an essential factor for the reduction.

3. An important factor responsible for calcium and acidity variations in gastric secretions is the presence of varying amounts of mucus in secretions of different types. Calcium is liberated from the mucus in a reaction associated with the reduction of acidity and is an index of this reaction.

4. The opacity of "visible" mucus is due to the presence of large numbers of mucus-secreting cells which secrete the alkaline mucus in which they are suspended.

It is a pleasure to acknowledge the advice received from Prof. B. P. Babkin on many points during the course of this work.

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EXCITABILITY CYCLE OF THE HYPOTHALAMUS-SYMPATHETIC NEURONE SYSTEM

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In the course of a study of excitation of sympathetic neurones by hypothalamic stimulation (Pitts, Larrabee and Bronk, 1941), mechanisms were revealed for the gradation of peripheral sympathetic responses similar to those described by Adrian and Bronk (1928) for control of somatic motor reflexes, and by Bronk and Ferguson (1935) and Gesell, Atkinson and Brown (1941) for control of respiration. Magnitude of response in all three instances is graded by two mechanisms: variation in the frequency of repetitive activity of any given single neurone and variation in the number of neurones active.

Simple relationships were found between the frequency of firing of cervical sympathetic neurones and the frequency and intensity of hypothalamic stimulation. These relationships suggest that a number of parallel pathways descend from the hypothalamus through the brainstem, to make synaptic connection with a given preganglionic efferent neurone. The average level of excitation of the system, measured by the frequency at which the efferent neurone fires, is dependent on the number of pathways within the sphere of influence of the stimulus and the frequency at which these pathways are stimulated. However, each response of the neurone follows, with relatively fixed latency, a particular stimulus to the hypothalamus, and is therefore triggered by a particular volley of impulses.

Qualitatively, these observations are remarkably similar to those of Bronk (1939) and Bronk and Larrabee (in preparation) for excitation of postganglionic neurones of the stellate ganglion by preganglionic stimulation. Despite the enormous differences in morphological complexity between the ganglionic system, the somatic motor reflex arc, the respiratory system and the central sympathetic projection systems, the similarities pointed out suggest that some fundamental factors are common to them all. The ganglionic system, being the least complex, is the better understood. Consequently it was felt that if experiments analogous to those performed on the ganglion could be applied to the central sympathetic system, information might be obtained which could be interpreted on the basis of known behavior of the simpler system.

In the previous paper it was shown that a single neurone dissected from the

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cervical sympathetic nerve of one side could be caused to fire by repetitive stimulation of either the same or the opposite side of the hypothalamus. This fact has enabled us to study summation of excitation of a single neurone brought about by volleys of impulses from two different sources. Furthermore, it has enabled us to analyze changes in excitability produced by volleys of variable intensity, frequency and duration from one source, in terms of the response to constant volleys from another source. The present paper is a presentation of this analysis.

METHODS. Experiments were performed on cats anesthetized with 30 mgm. per kilo of sodium delvinal given intravenously. Two concentric bipolar needle electrodes, insulated except at their tips, were oriented by means of the Horsley-Clarke stereotaxic instrument in the tuberal portion of the hypothalamus, 2 mm. to either side of the mid line. At the end of each experiment the brain was perfused with formalin, removed and allowed to harden. They were embedded in nitrocellulose, sectioned at 50 microns, stained by the Weil method and examined to determine the placement of the electrode tips. Except for slight deviations in two experiments, the electrodes were found to be quite symmetrically placed.

Two identical thyratron controlled stimulators, whose frequencies and intensities were independently variable, were used for stimulation of the two sides of the hypothalamus. The maximum intensity used was below that which leads to any significant motor response. The frequency range of the stimulators lay between 1 and 180 stimuli per second. Strands, which contained but a single functional nerve fiber, were dissected from the cervical sympathetic trunk in the neck. The dissection was performed in a small moist chamber in a shielded room maintained near 37°C. and 100 per cent humidity. Fibers from right and left sympathetic trunks were used. Since no differences between the two were noted, the side of the hypothalamus stimulated will be designated merely as ipsilateral or contralateral to the sympathetic nerve recorded from. Action potentials of single fibers were amplified by a capacity coupled amplifier having a time constant of 0.1 second and were recorded with a General Electric oscillograph and a moving bromide paper camera.

RESULTS. *Summation of excitation from the right and left hypothalamus.* Stimulation of the hypothalamus with brief repetitive condenser discharges of low intensity and high frequency causes cervical sympathetic neurones to fire rhythmically and repetitively at a frequency which is considerably less than the stimulus frequency (Pitts, Larrabee and Bronk, 1941). After an initial period during which frequency declines, the rate of firing becomes relatively constant. These adapted response frequencies for any given neurone are repeatable if a half minute or more intervenes between periods of stimulation.

If two pairs of electrodes are introduced into the hypothalamus approximately 2 mm. to either side of the mid line with their exposed tips at the level of the fornix, as shown in figure 1, stimulation through either pair of electrodes excites a given neurone and causes it to fire. Figure 2 shows the response of a single neurone to stimulation of both sides of the hypothalamus. In

record *A*, the hypothalamus ipsilateral to the nerve recorded from was stimulated with low intensity shocks at a frequency of 175 per second. The neurone fired at a frequency of 10.8 per second, once to approximately every sixteenth stimulus. In record *B*, the contralateral hypothalamus was stimulated with the same frequency and intensity of shocks, the neurone firing 10.5 times per second. In record *C* both sides were stimulated simultaneously and the response frequency approximately doubled to 20.4 per second. Record *D* shows initially the result of stimulation of the ipsilateral hypothalamus, and after the arrow, the result of stimulation of both sides.

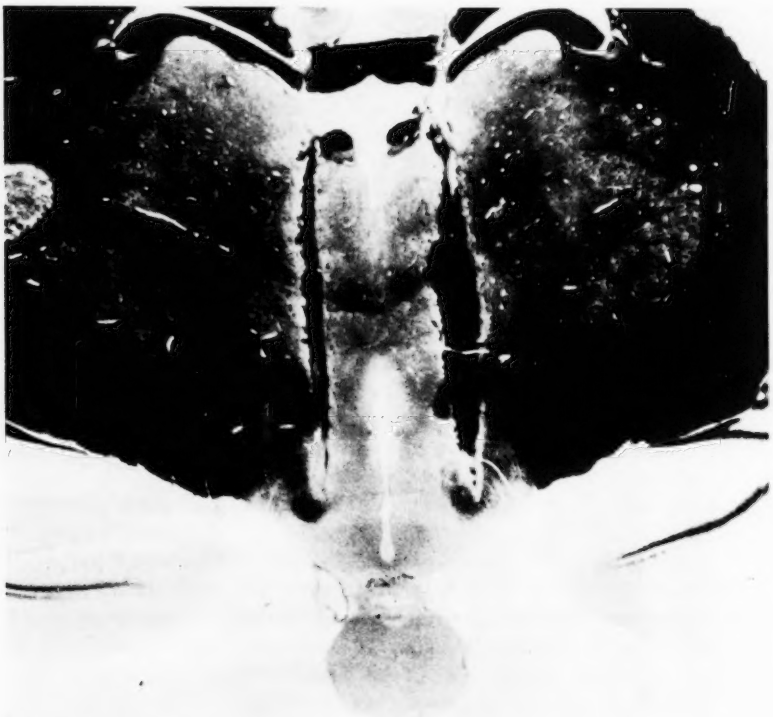

Fig. 1. Photomicrograph of a Weil stained section through the diencephalon of the cat to show the position of the two pairs of stimulating electrodes, 2 mm. to either side of the midline. The exposed tips of the electrodes lay within the lateral hypothalamic area at the level of the fornix. For results of stimulation, see figure 2.

In 14 experiments in which the response of 43 different neurones has been studied, no instance has been found in which a neurone capable of being excited from one side of the hypothalamus could not also be excited from the other side. The threshold intensity of stimulation often differs slightly on the two sides and if definite differences are found, the threshold is lower on the ipsilateral side.

A small increase in intensity on the less excitable side, however, will always cause the neurone to fire. The response frequencies of a neurone to stimulation of the two sides of the hypothalamus with identical stimuli are not always so nearly the same as those shown in figure 2. The frequency at which the neurone fires is commonly greater on stimulation of the ipsilateral hypothalamus, never less.

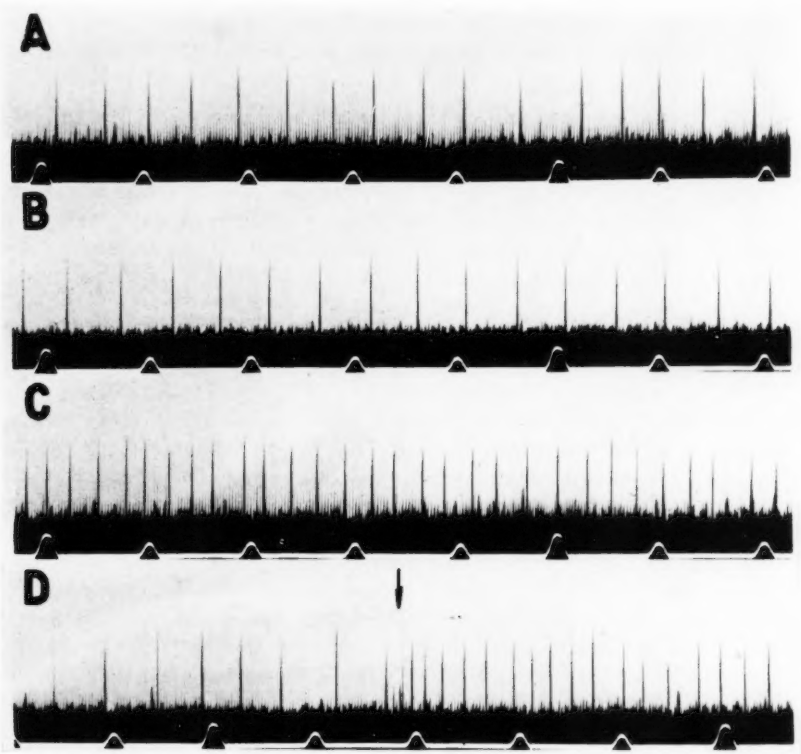

Fig. 2. The discharge of impulses by a single neurone of the left cervical sympathetic nerve on stimulation of the left and right sides of the hypothalamus. For electrode position, see figure 1. *A*, stimulation of left hypothalamus; *B*, stimulation of right hypothalamus; *C*, simultaneous stimulation of both; *D*, stimulation of first the left and at the arrow both the left and right sides of the hypothalamus. Time, $\frac{1}{5}$ second.

Almost perfect summation is shown in figure 2 for this neurone at the frequency and intensity of stimulation used. The summated response is only 4 per cent less than the arithmetic sum of the individual responses. However, depending on the frequency and intensity of stimulation used, the summated frequency of firing may be greater than, equal to or less than the sum of the individual frequencies. Occlusion, i.e., a summated response less in magnitude

than the sum of the individual responses, has never been observed to be greater than 15 per cent.

Summation at different stimulus intensities. It was observed by Pitts, Larrabee and Bronk (1941) that the frequency of firing of single sympathetic neurones increases with an increase in intensity of hypothalamic stimulation. The relation between frequency of response and stimulus intensity is smoothly curved and sigmoid in form, with a tendency for the response frequency to level off at higher stimulus intensities. The essential character of this relationship was found to hold in a number of experiments, independent of the peak response frequency attained or of the frequency of hypothalamic stimulation. The nature of the curve suggests that the limitation of frequency of firing at the higher intensities might result from activation of the entire excitable area of the hypothalamus which is in synaptic relation with a given neurone. Against this view are the facts that the maximum stimulus intensities were low, the exposed tips of the electrodes were small and close together and the excitable area of the hypothalamus for a given neurone quite large. The results obtained by summating excitation from the two sides of the hypothalamus at different stimulus intensities make the view completely untenable.

If the two sides of the hypothalamus are stimulated separately and then simultaneously with constant frequency (180 per sec.) and various intensities of shocks, the relationship between frequency of firing of a single neurone and intensity of stimulation may be determined for ipsilateral, contralateral and summated hypothalamic excitation. Figures 3A and B show plots of the data so obtained from two experiments. The two experiments are presented to show that whether the response frequencies from the two sides of the hypothalamus are the same or different at any given intensity, the essential features of the curves are unmodified.

It is evident that the relationship between the number of impulses discharged per second by a given preganglionic neurone and the intensity of hypothalamic stimulation is qualitatively the same for excitation of each of the two sides of the hypothalamus, though quantitatively it may or may not be less for the contralateral side. Furthermore, the relation is similar when both sides of the hypothalamus are stimulated simultaneously, though of course the frequencies are much greater. Closer inspection of the figures reveals various degrees of summation. Figure 3B, for example, illustrates at intensity 2, summation of subliminal degrees of excitation, while at intensities of 3, 5 and 8 occlusion is evident. In both experiments the highest intensity used is that at which somatic motor responses begin.

It is evident that the leveling off of the curves at the higher intensities is not a result of current spread which excites the entire hypothalamus, for excitation of both sides simultaneously at the maximum intensities still leads to summation with very little occlusion. Lacking knowledge of relative thresholds and densities of fibers and cells about the electrodes, explanation of the form of the curves is impossible.

Summation at different stimulus frequencies. If the above type of experiment

is repeated maintaining intensity of stimulation constant and varying the frequency, the results obtained cast further light on the process of summation. It was noted previously (Pitts, Larrabee and Bronk, 1941) that the relationship between frequency of firing of sympathetic neurones and frequency of hypothalamic stimulation is a linear one over a considerable part of the frequency range. As shown in figure 4, this relationship holds whether stimuli are applied to the same or the opposite side of the hypothalamus. The summated responses are also linearly related to the stimulus frequency but are plotted differently

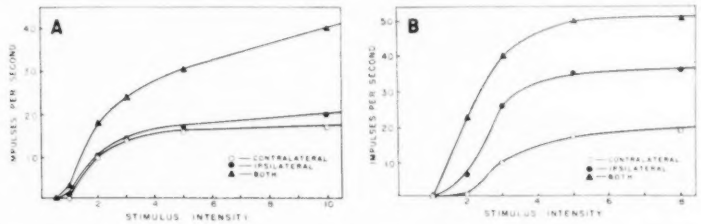

Fig. 3. The relation between frequency of discharge of impulses in single fibers of the cervical sympathetic nerve and intensity of stimulation applied to the two sides of the hypothalamus singly and together. *A* and *B*, separate experiments. Stimulus frequency, 180 per second throughout.

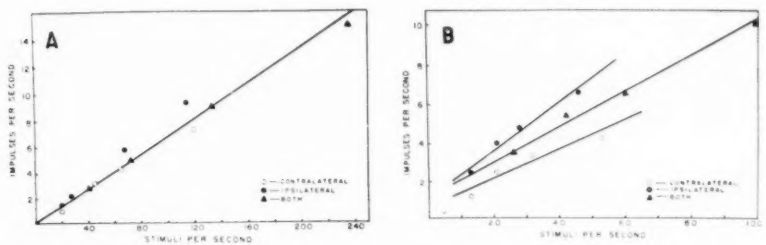

Fig. 4. The relation between frequency of discharge of impulses in single fibers of the cervical sympathetic nerve and frequency of stimuli applied to the two sides of the hypothalamus singly and together. *A* and *B*, separate experiments. Stimulus intensity the same on the two sides and constant throughout. Note that the summated responses are plotted against the sum of the stimulus frequencies applied to the two sides.

from those of the preceding figure to emphasize a striking feature of summation. The summated response frequency obtained on stimulation of the two sides simultaneously is plotted against the *sum* of the two stimulus frequencies. The summated responses then fall upon a line which constitutes a mean of the responses of the two sides separately. Roughly, the relation holds whether ipsilateral and contralateral responses to a given stimulus are nearly the same (fig. 4A) or are quite different (fig. 4B).

It is evident that whatever the mechanism is which determines the frequency of firing of the final sympathetic neurone, it is activated to approximately the same degree by volleys of impulses of a given frequency carried simultaneously

by two separate pathways or by volleys at twice that frequency carried by either of the pathways singly. This can only be true if occlusion between the two pathways is minimal and if a given pathway is working safely below the top frequency at which it can transmit volleys. It is clear also from the preceding experiments (figs. 3A and B) that doubling the intensity of stimulation on one side of the hypothalamus does not produce a response equivalent to that of a given intensity simultaneously applied to the two sides. Hence doubling stimulus intensity must fall short of doubling the number of separate pathways connecting the hypothalamus with the frequency determining mechanism. The result cannot be explained on a basis of greater occlusion at higher stimulus intensities for such occlusion is minimal.

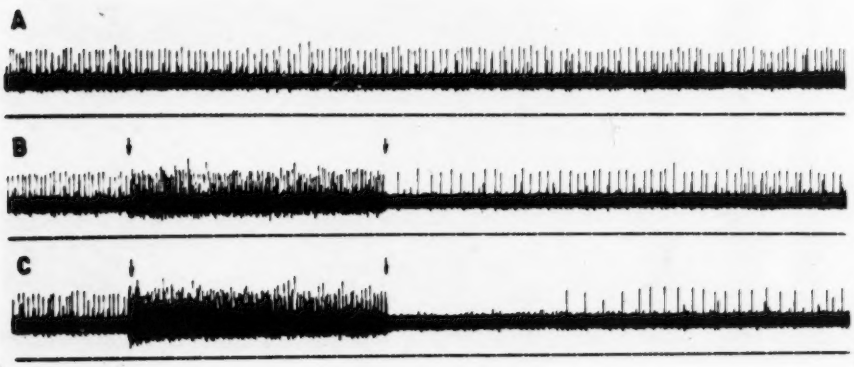

Fig. 5. The effect of a brief period of stimulation of the ipsilateral hypothalamus on the discharge frequency of a fiber of the cervical sympathetic nerve activated by maintained stimulation of the contralateral hypothalamus. *A*, control record, stimulation of the contralateral hypothalamus only; *B* and *C*, same contralateral stimulation and in addition ipsilateral stimulation for the 3 second interval marked off by arrows. *B*, stimulus frequency 100 per second; *C*, 150 per second. Time, ½ second.

Reduction of excitability as a result of activity. The fact, demonstrated above, that a single sympathetic neurone may be excited through two sets of pathways which show little occlusion makes it possible to study changes in excitability of the hypothalamic-sympathetic neurone system at or below the point of convergence of the pathways, independent of changes in the separate pathways themselves. That changes in excitability do occur was noted early in our work when it was found that periods of stimulation of the hypothalamus, repeated at short intervals, led to response frequencies which progressively declined. It was found that intervals of a half minute or more between periods of stimulation allowed adequate recovery and routinely we have used recovery periods of 1 minute or more.

Figure 5 illustrates the essential features of the excitability changes which we shall analyze in the following sections. The records were made with a

cathode ray oscillograph in order to record at slow enough paper speed to reproduce the major part of the experiment. Record *A* illustrates the response of a cervical sympathetic neurone to maintained stimulation of the contralateral hypothalamus with low intensity, high frequency shocks. In records *B* and *C* the same stimulation was maintained throughout but, in addition, during the interval marked off by the arrows, stimuli of low intensity were applied to the ipsilateral hypothalamus. In record *B* the frequency of ipsilateral stimulation was 100: in *C*, 150 per second.

In record *A*, contralateral stimulation caused the neurone, the response of which is readily identified, to fire repetitively at a frequency which was fairly well maintained. In *B* and *C* simultaneous stimulation of the ipsilateral hypothalamus raised the frequency of firing, more in *C* than in *B*, as a consequence of the more rapid stimulation. The change in the frequency of response of the neurone to the maintained contralateral stimulation after the period of summated response is very evident. Record *B* shows a slowing of the frequency of firing with a gradual return to the control value. In record *C*, however, in which the summated response was greater as a result of higher frequency hypothalamic stimulation (150 per sec.), the neurone ceased to fire for over 3 seconds, and then began again at a much lower frequency which was still depressed at the end of the record.

The general aspects of the picture of excitability change, presented in figure 5, duplicate almost exactly those noted by Bronk (1939, fig. 16) in the stellate ganglion. Bronk has shown that single postganglionic neurones may be caused to fire rhythmically and repetitively by perfusion of the stellate ganglion with Ringer's solution containing small amounts of acetylcholine. Stimulation of the preganglionic trunk with repetitive shocks increases the rate of firing of the neurone, and after the stimulus ceases the response frequency of the neurone drops below its control value or ceases entirely for a time depending on the frequency of the preceding stimulation. Bronk has correlated this reduced excitability after preganglionic volleys with the positive afterpotential of the ganglion. The duration and degree of reduced excitability, indicated by the frequency at which the neurone fires, is related to the duration and magnitude of the potential.

Gasser (1936) has pointed out that the excitability of peripheral nerve is decreased by repetitive shocks in proportion to the frequency or duration of stimulation. He has shown that a positive afterpotential parallels almost exactly the reduction in excitability, and has pointed out a possible explanation, in terms of excitability change and afterpotential in the spinal cord, for the silent period of extensor tone following elicitation of the knee jerk.

The experiments of Gasser and Bronk suggest that a similar mechanism may account for the reduced excitability observed in our experiments. Accordingly, we have explored excitability changes in the hypothalamic-sympathetic neurone system after stimulation of the hypothalamus with stimuli of different intensities and frequencies and for periods of variable duration.

Factors involved in determining the degree of reduction in excitability. The same

methods of quantitation which were used in determining summation of excitation have been applied in experiments of the type just described to determine changes in excitability which result from hypothalamic stimulation. In order to measure intervals between the nerve impulses accurately, records were taken at higher speed than those shown in figure 5. We have found a paper speed of 100 mm. per second adequate for measurement and the records which we have analyzed are similar to those shown in figures 9 and 11.

The experiments have all been performed in a similar manner. The contralateral hypothalamus has been stimulated at such an intensity and frequency that the cervical sympathetic neurones examined have been caused to fire at some frequency between 6 and 12 per second. An initial and a final control

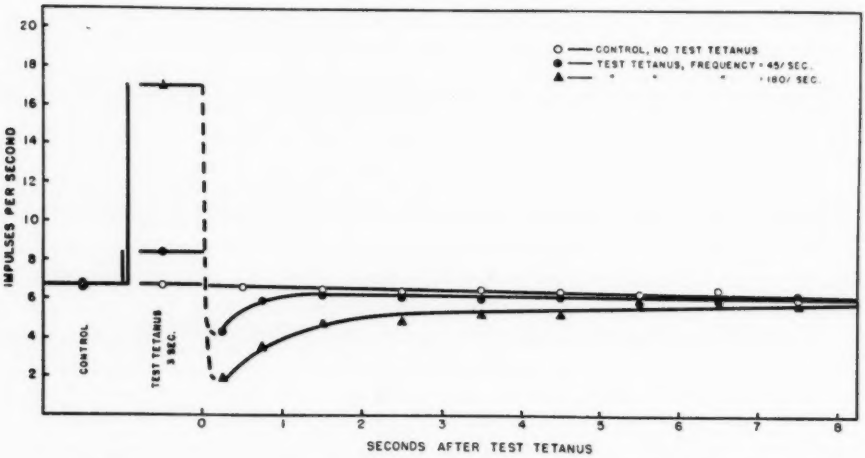

Fig. 6. Summation of excitation during, and recovery of excitability after 3 second periods of stimulation of the ipsilateral hypothalamus at frequencies of 45 and 180 per second. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

period of stimulation of the contralateral hypothalamus for some 20 seconds provides a base line of reference. This same stimulation is maintained throughout all succeeding records. After an initial control period of 3 seconds during which only the contralateral hypothalamus is stimulated, there follows a 3 second testing period in which the ipsilateral hypothalamus is also stimulated with shocks of varying intensity or frequency. The succeeding 10 second period of the record, during which only the contralateral hypothalamus is stimulated permits an estimation, relative to the control, of changes in excitability resulting from the testing period of ipsilateral stimulation.

A. *Effect of frequency of test stimulation.* In figure 6 is presented an analysis of such an experiment, in which changes in excitability resulting from 3 second tests at frequencies of 45 and 180 shocks per second are compared. The refer-

ence base line indicates that the neurone maintained a fairly constant frequency of firing to maintained stimulation of the contralateral hypothalamus. This frequency is also shown to have been identical at the start of each of the two succeeding tests; i.e., all 3 control frequencies superimpose. During the 3 second test period of stimulation of the ipsilateral hypothalamus at 45 per second, the firing of the neurone increased but slightly, and following this period, fell but little below the control valve and rapidly approached it again. During stimulation of the ipsilateral hypothalamus at 180 per second, the frequency of firing of the neurone was considerably elevated (2.5 times). Following stimulation, the frequency was depressed initially and returned quite slowly to the control value.

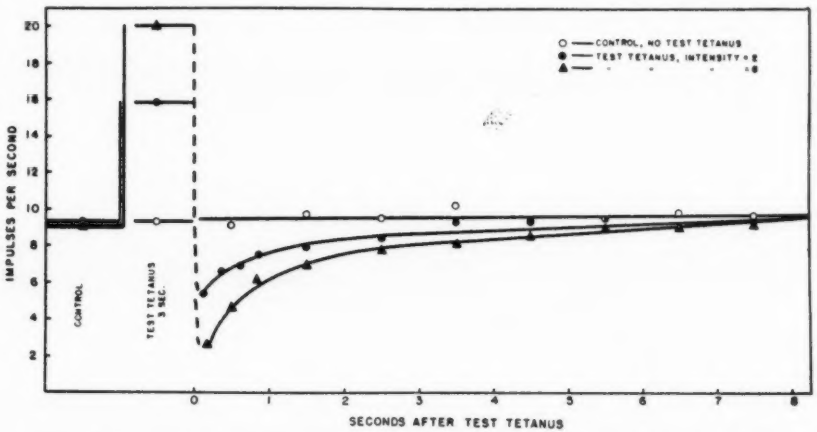

Fig. 7. Summation of excitation during, and recovery of excitability after 3 second periods of stimulation of the ipsilateral hypothalamus at intensities of 2 and 8. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

B. *Effect of intensity of test stimulation.* Identical results have been obtained on comparing changes in excitability produced by test stimuli of varying intensity. In figure 7 is presented one such experiment, in which the frequency of stimulation in both instances was 150 per second, but the intensities 2 and 8. There was a greater summated increase in response to intensity 8 than to intensity 2, and following the period of stimulation a greater initial depression of excitability which was maintained for a longer time. It is evident that an increase in frequency of stimulation at constant intensity or an increase in intensity at constant frequency not only produces a greater summated response during stimulation and a greater initial depression of excitability after stimulation, but also maintains the excitability at a depressed level for a longer period of time.

C. *Effect of duration of test stimulation.* Figure 8 presents an analysis of the

methods of quantitation which were used in determining summation of excitation have been applied in experiments of the type just described to determine changes in excitability which result from hypothalamic stimulation. In order to measure intervals between the nerve impulses accurately, records were taken at higher speed than those shown in figure 5. We have found a paper speed of 100 mm. per second adequate for measurement and the records which we have analyzed are similar to those shown in figures 9 and 11.

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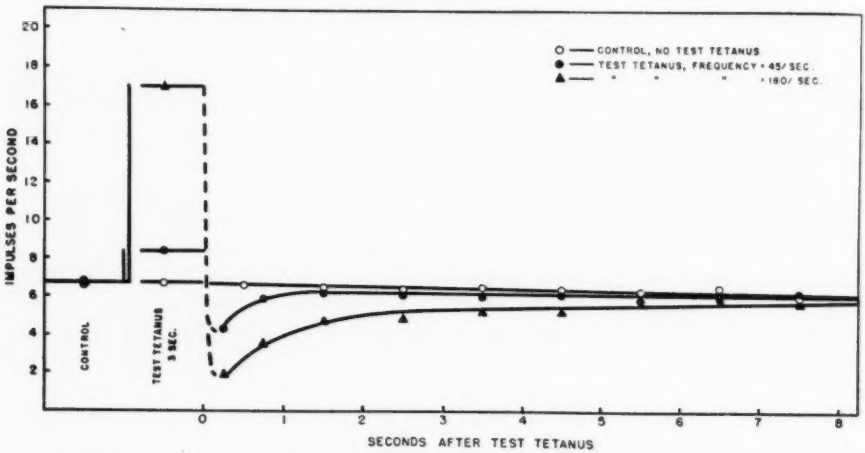
CONTROL, NO TEST TETANUS
TEST TETANUS, FREQUENCY = 45/ SEC.
" " " = 180/ SEC.
IMPULSES PER SECOND
CONTROL
TEST TETANUS 3 SEC.
SECONDS AFTER TEST TETANUS

Fig. 6. Summation of excitation during, and recovery of excitability after 3 second periods of stimulation of the ipsilateral hypothalamus at frequencies of 45 and 180 per second. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

period of stimulation of the contralateral hypothalamus for some 20 seconds provides a base line of reference. This same stimulation is maintained throughout all succeeding records. After an initial control period of 3 seconds during which only the contralateral hypothalamus is stimulated, there follows a 3 second testing period in which the ipsilateral hypothalamus is also stimulated with shocks of varying intensity or frequency. The succeeding 10 second period of the record, during which only the contralateral hypothalamus is stimulated permits an estimation, relative to the control, of changes in excitability resulting from the testing period of ipsilateral stimulation.

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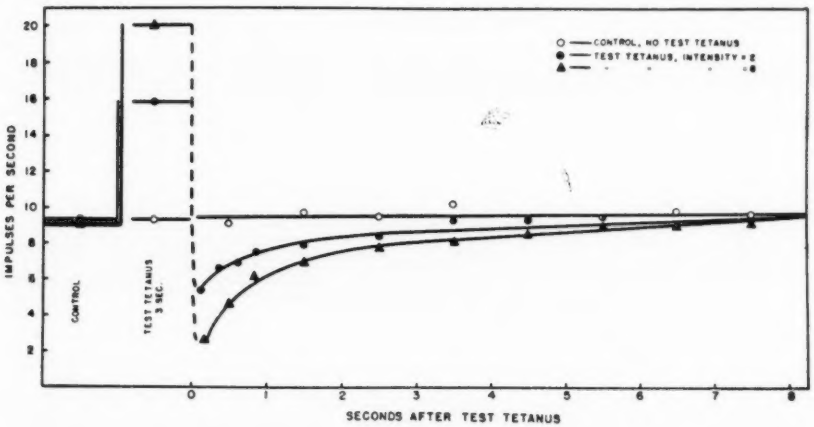

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B. *Effect of intensity of test stimulation.* Identical results have been obtained on comparing changes in excitability produced by test stimuli of varying intensity. In figure 7 is presented one such experiment, in which the frequency of stimulation in both instances was 150 per second, but the intensities 2 and 8. There was a greater summated increase in response to intensity 8 than to intensity 2, and following the period of stimulation a greater initial depression of excitability which was maintained for a longer time. It is evident that an increase in frequency of stimulation at constant intensity or an increase in intensity at constant frequency not only produces a greater summated response during stimulation and a greater initial depression of excitability after stimulation, but also maintains the excitability at a depressed level for a longer period of time.

C. *Effect of duration of test stimulation.* Figure 8 presents an analysis of the

changes in excitability which result from stimulation with shocks of constant frequency and intensity for variable lengths of time. It is apparent that the initial depression of excitability after stimulation is but little altered by duration of the stimulation period. But the time that excitability remains depressed is much greater when stimulation is maintained 3.5 seconds than when it lasts but 0.2 second. To this extent there is a difference between the excitability changes resulting from increased intensity or frequency of stimulation and increased duration, for the former show variations in initial depression of excitability, whereas the latter do not.

No supernormal phase of excitability is evident in any of our experiments, all of which have been repeated several times. Furthermore, none is found in peripheral nerve subjected to repetitive stimulation under conditions similar to those

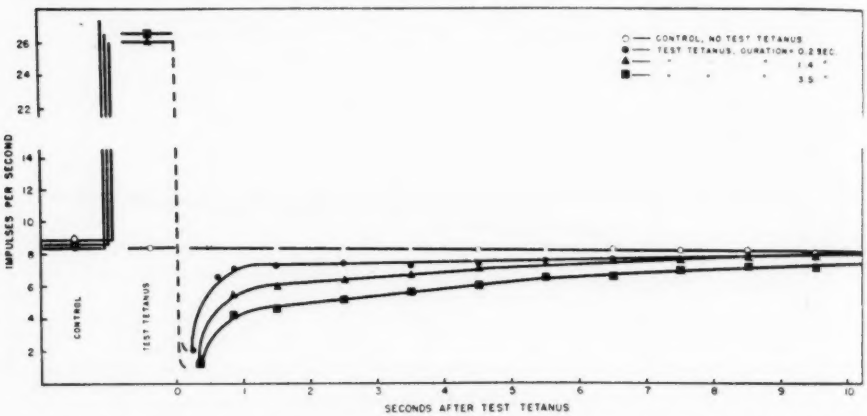

Fig. 8. Summation of excitation during, and recovery of excitability after stimulation of the ipsilateral hypothalamus for periods of 0.2, 1.4 and 3.5 seconds. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

above. The fact that the depression of excitability in both peripheral nerve and ganglia has been correlated with a positive afterpotential tempts one to carry over the association to the central system studied here. However, such potentials if they exist, have not been recorded.

Independence of excitability depression and rise in blood pressure. The possibility exists that the depressed excitability noted after the test period of stimulation in reality represents sympathetic inhibition as a result of a rise in blood pressure and excitation of pressure sensitive afferents. Inhibition of spontaneous sympathetic discharge in the inferior cardiac nerve as a result of a rise in blood pressure produced by a brief period of hypothalamic stimulation has been described by Pitts, Larrabee and Bronk (1941). Furthermore it has been shown that a rise in blood pressure produced by intravenous adrenalin causes slowing and eventual cessation of the discharge of single neurones of the cervical sympathetic caused to fire at a very low frequency by low intensity hypothala-

mic stimulation. However, it may readily be shown that under the conditions of the experiments just reported no essential part of the decreased excitability following activity results from inhibition in consequence of excitation of pressure afferents by a rise in blood pressure.

In figure 9 is shown a repetition of one of these experiments with a simultaneous record of blood pressure. In records *A*, *B* and *C* the contralateral hypothalamus was stimulated with low intensity, high frequency stimuli. During the 3 second interval marked off by the arrows, stimuli at a frequency

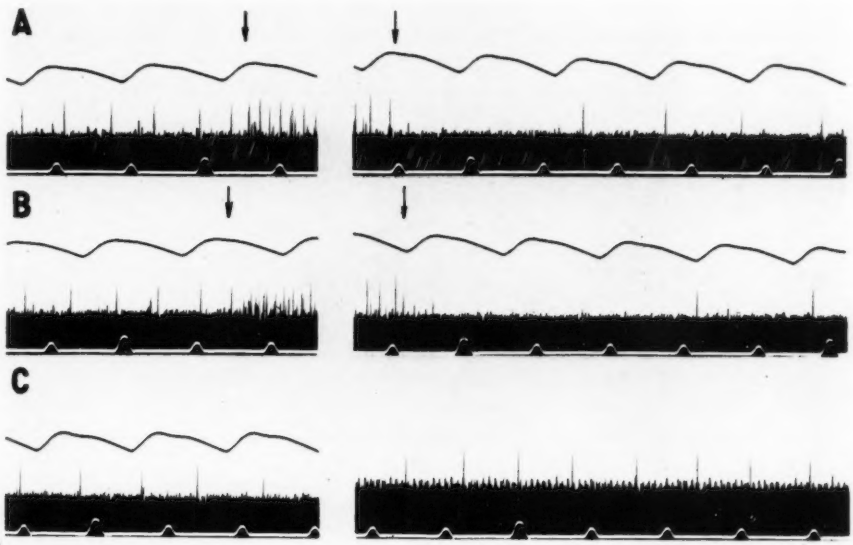

Fig. 9. Comparison of the effects of ipsilateral hypothalamic stimulation and a rise in blood pressure induced by intravenous adrenalin on the discharge of a single fiber of the cervical sympathetic nerve activated by maintained stimulation of the contralateral hypothalamus. *A* and *B*, stimulation of the ipsilateral hypothalamus for the 3 second interval between the arrows, at frequencies of 100 and 150 per second. *C*, adrenalin intravenously during the break in the record; blood pressure elevated from 11 to 250 mm. Hg. and did not record on the paper. Time, $\frac{1}{5}$ second.

of 100 per second (record *A*) and 150 per second (record *B*) were applied to the ipsilateral hypothalamus. In record *C*, adrenalin was administered intravenously. Records *A* and *B* show a decreased excitability following the test period of hypothalamic stimulation proportional to the frequency of stimulation, with little elevation of blood pressure. Record *C* shows essentially no change of excitability as a result of more than a two-fold elevation of blood pressure, so great that the tracing failed to record on the paper. It is evident that a rise in blood pressure, per se, does not explain the diminished excitability which we have noted under these experimental conditions.

Site of the depression of excitability. The changes in excitability which we have described after a test period of hypothalamic stimulation are evidently located somewhere at or below the point of convergence of pathways from the two sides of the hypothalamus. Thus a brief test period of stimulation to one side can affect the excitability to a maintained stimulus to the other side only at some point common to the two pathways. It is evident also that a change of excitability in either pathway before convergence can be revealed only if it can be made greater than the change in excitability after convergence. If such changes in excitability in the individual pathways before convergence are of anywhere near the same order of magnitude as those after convergence, it should be possible by increasing the volley load of one pathway to bring out those changes.

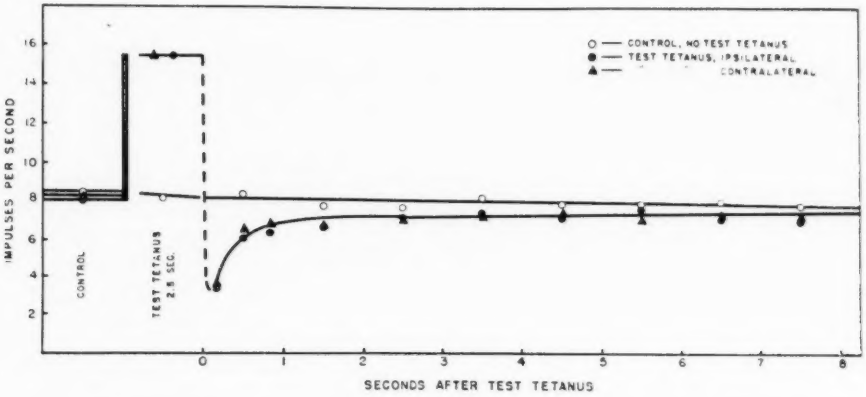

Fig. 10. Summation of excitation during, and recovery of excitability after identical testing periods of stimulation to the ipsilateral and to the contralateral sides of the hypothalamus. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

To this end we have compared the effects of applying the test stimuli to the same pathways which carry the constant volley load, with the effects of applying them to the inactive pathways descending from the opposite side of the hypothalamus. Figure 10 shows the result in one such experiment. The degree of depression of excitability after the test period of stimulation was identical whether the testing volleys were carried by the same pathways as carried the constant volleys or by pathways descending from the opposite hypothalamus. In this experiment, and in another yielding the same result, the discharge of the sympathetic neurone was increased to an identical extent by the test stimulation whether applied ipsilateral or contralateral to the constant stimulus. Three other experiments, however, differed from these, all in the same way. Test stimulation of the side of the hypothalamus opposite to that to which the constant stimuli were applied, caused the greatest increase in firing of the preganglionic neurone, and after stimulation, the greatest depression in excitability.

It is evident from these experiments that an increase in the volley load of one pathway above the convergence point cannot cause a depression of excitability of that pathway which is equivalent to the depression produced further down. Whether or not this means that the pathways down to the point of convergence are formed of axons of single neurones without any interposed synapses is not certain. It does mean, however, that depression of excitability at such synapses, if there are any, is not of the same order of magnitude as that which occurs farther down. The experiments suggest that the point of depression of excitability may be the same as the point at which the response frequency of the final neurone is determined. In all our experiments there is a direct relation between frequency of firing of the final neurone and the degree of depression which follows. The most obvious site at which both the limiting depression of excita-

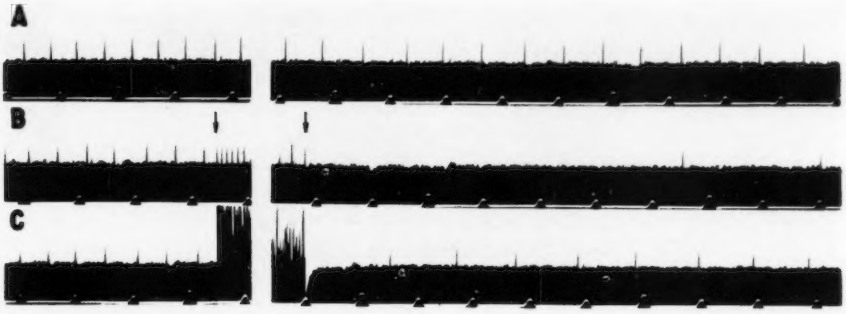

Fig. 11. Comparison of the effects of dromic and antidromic stimulation on the discharge frequency of a single fiber of the cervical sympathetic nerve activated by maintained stimulation of the contralateral hypothalamus. *A*, control record, stimulation of the contralateral hypothalamus only. *B* and *C*, same contralateral hypothalamic stimulation throughout. *B*, ipsilateral hypothalamic stimulation at 170 per second; *C*, ipsilateral antidromic stimulation of the cervical sympathetic nerve at 170 per second. Time, $\frac{1}{5}$ second. For complete data see figure 12.

bility might occur and the response of the final neurone be determined, is at the final preganglionic neurone itself.

Some evidence (Eccles and Sherrington, 1931) points to the fact that an impulse conducted antidromically passes from axon to soma of a neurone and produces changes in excitability similar to those produced when the neurone fires in response to presynaptic excitation. If this is true, antidromic stimulation of the sympathetic neurone at a frequency comparable to that at which it is caused to fire by the testing hypothalamic stimulation, should produce a comparable depression of excitability if the preganglionic neurone is the site at which depression occurs.

In figures 11 and 12 records of one such experiment are presented. Record *A* in figure 11 shows the firing of a single neurone in response to maintained stimulation of the contralateral hypothalamus. The same stimulation was main-

tained throughout records *B* and *C*. In record *B*, during the 5 second interval indicated between the arrows, the ipsilateral hypothalamus was stimulated with low intensity, high frequency stimuli. The frequency of firing of the neurone was raised from a control value of 10 per second to 22 per second. In record *C* the neurone was stimulated antidromically for 5 seconds at a frequency of 170 per second. It is quite evident that hypothalamic stimulation, producing an increase in response from 10 to 22 impulses per second, is followed by a much greater depression in excitability than is antidromic stimulation at a rate some 8 times as great.

The complete data of this experiment are shown in figure 12. Antidromic stimulation at 27 per second, comparable to the 22 per second discharge in-

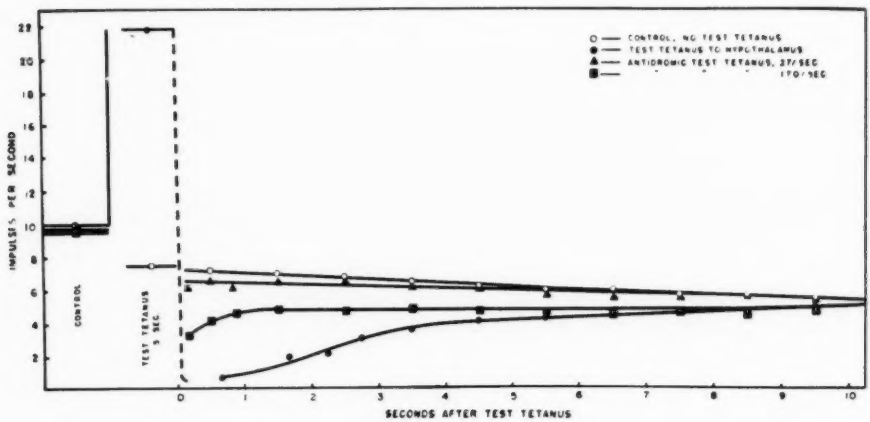

Fig. 12. Summation of excitation during, and recovery of excitability after dromic (ipsilateral hypothalamic) and antidromic (cervical sympathetic nerve) stimulation of a single cervical sympathetic neurone. Stimulation of the contralateral hypothalamus constant throughout. See text for details.

duced by dromic stimulation, produced a scarcely detectable depression in excitability. Moreover, the depression in excitability resulting from antidromic stimulation at 170 per second does not equal that produced by the lower frequency hypothalamic stimulation. If we accept the thesis that dromic and antidromic excitation lead to comparable changes in excitability, then the site of major depression following hypothalamic stimulation, though at or caudal to the point of convergence of pathways from the two sides of the hypothalamus, nevertheless lies cephalic to the final common sympathetic neurone. Such a view is of course based upon what may be an erroneous assumption. It is not the outcome anticipated.

Discussion. The results which have been presented in the preceding pages should most logically be discussed in terms of the anatomical makeup of the connecting system between hypothalamus and preganglionic sympathetic

neurone. But detailed knowledge of these connections is lacking. Such information as is available is derived chiefly from physiological experimentation, and in place of a desired rigorous analysis of function in terms of known structure, one is forced to infer structure from function.

It is generally conceded that the lateral and posterior hypothalamic areas form a homogeneous system, the activation of which leads to widespread sympathetic discharge (Ranson and Magoun, 1939; Ingram, 1939). The degree of homogeneity of the hypothalamus is emphasized in a very real manner by the demonstration that single neurones of the cervical sympathetic may be excited by stimulation of a fairly extensive area not only on the same side of the hypothalamus, but on the opposite side as well.

The pathways connecting the hypothalamus with preganglionic sympathetic neurones, in so far as they are known, have been described by Magoun (1940) and Wang and Ranson (1939a and b). They descend from the lateral hypothalamic areas through both the central grey and tegmentum of the mesencephalon into the pons, where they are concentrated chiefly in the tegmental region. In the medulla they pass caudally in the more lateral portions of the reticular formation through the region of the myelencephalic sympathetic center (Ranson and Billingsley, 1916; Chen, Lim, Wang and Yi, 1936; 1937). In the spinal cord they are located in the anterolateral funiculus. The pathways are made up of both crossed and uncrossed connections at every level, for decussation appears to be progressive throughout the brainstem, perhaps even in the cord. Whether and where these pathways may be interrupted in their descending course is unknown, although the nature of their decussations suggests that single axons could scarcely accomplish the manoeuvers. Little has been added by our study to the general topographical knowledge of the descending hypothalamic connections, but certain implications as to their nature are evident. It is apparent that each preganglionic neurone of the sympathetic system is in functional connection with many different regions within the hypothalamus, not only on the same but also on the opposite side. The corollary, that each point within the hypothalamus is in functional connection with many preganglionic neurones on both sides of the cord, is obviously equally true. Such functional interrelation between wide areas of the hypothalamus and many preganglionic neurones implies either unreasonable multiplicity of connections or some system upon which excitation converges from both sides of the hypothalamus and from which excitation is distributed to sympathetic motorneurones on both sides of the cord. Possibly the medullary sympathetic center plays such a rôle.

In the light of the evident complexity of the hypothalamic-preganglionic neurone system, it is remarkable that it shows in simple form many of the properties of spinal cord, ganglion and even peripheral nerve. It has been shown (Pitts, Larrabee and Bronk, 1941) that an excitatory state, built up by successive volleys of impulses from the hypothalamus, reaches a critical level at which a single detonator volley triggers the system and causes an impulse to be discharged. An increase in rate of stimulation, increasing the number of

volleys carried by the pathways, or an increase in intensity of stimulation, increasing the number of active pathways, leads to an initial earlier attainment of the critical excitatory level, and to the maintenance of a higher average level of excitation as well. It was suggested that the frequency of firing of a given preganglionic neurone is determined, once the neurone has been caused to fire, by a balance between the rate of recovery of excitability and the average maintained level of excitation at some critical point within the system.

The present paper contributes to our knowledge of both variables which are presumed to determine the frequency of sympathetic response. The activation of pathways descending from each side of the hypothalamus contributes to the excitatory state which determines the rate of firing of a given sympathetic neurone. Simultaneous activation of these pathways increases the excitatory state to such a degree that the frequency of firing of the neurone may be greater than, equal to, or slightly less than the sum of the frequencies when the paths are activated separately. The frequency determining mechanism for the sympathetic neurone shows the classical properties of the spinal reflex arc, summation of subliminal excitation, arithmetic summation, and occlusion.

The other determinant of the frequency at which a preganglionic neurone fires, namely, recovery of excitability, has been shown to be influenced by those same factors which control recovery in peripheral nerve (Gasser, 1936) and sympathetic ganglia (Bronk, 1939). Following a period of repetitive stimulation, excitability is depressed in proportion to the frequency and intensity of the stimuli, and is prolonged in proportion to these same variables and to the additional one of duration. In peripheral nerves and ganglia the changes in excitability are paralleled by positive afterpotentials. The central excitability changes may well be accompanied by potential variations of similar sign, though they have not been recorded so far. But the fundamental mechanisms, of which excitability change and after potential are an index, may be the same in the central system described here and in peripheral nerves and ganglia.

An unexpected outcome of our work has been the site at which the limiting factors, i.e., recovery of excitability and level of excitatory state, operate in determining frequency of firing of the preganglionic neurone. One would expect both factors to operate at the site of the preganglionic neurone itself, and such may well be the case. However, if it is correct to assume that antidromic stimulation of a neurone produces the same excitability changes that are produced when a neurone fires in response to presynaptic excitation (Eccles and Sherrington, 1931), then the preganglionic neurone cannot be the limiting site at which frequency is determined. The degree of depression of excitability after hypothalamic stimulation is much greater than after antidromic stimulation at a frequency comparable to that at which the neurone is caused to fire by the dromic excitation. Since the limiting site of frequency determination is inaccessible to antidromic stimulation, it must lie cephalic to the preganglionic neurone.

An experiment described by Pitts, Larrabee and Bronk (1941) suggests that

the frequency limiting system may lie at the level of the medulla oblongata. If single preganglionic neurones are caused to fire at low frequencies by very low intensity hypothalamic stimulation, a small rise in blood pressure causes a slowing of the rate of firing, while a greater rise inhibits the discharge completely. The buffer afferents are able to control the frequency of firing of preganglionic neurones to hypothalamic stimulation presumably at the level of the myelencephalic sympathetic center. It may well be at this same level that the limiting factors mentioned above operate in determining the frequency of firing of the final preganglionic neurone. The rest of the system, i.e., the spinal pathways and efferent preganglionic neurones, according to this concept, are working well below their limiting capacities and serve only to translate the medullary excitation into efferent sympathetic activity.

The nature of the excitatory state has not been implied in any way in the above discussion, and any consideration of its nature would be unjustified. It is well to point out that it is possible to account for our results equally well on the basis of any of the current concepts presumed to govern excitation at synapses. The depression of excitability following activity has been inferred to result from those same factors which depress excitability in sympathetic ganglia and in peripheral nerve. Other explanations, however, might well account for it.

SUMMARY

Stimulation of either side of the hypothalamus with low intensity, high frequency shocks causes preganglionic neurones of the cervical sympathetic to fire rhythmically and repetitively at frequencies proportional to the intensity and frequency of stimulation. If both sides of the hypothalamus are stimulated simultaneously, the neurones fire at higher frequencies which may be greater than, equal to or slightly less than the sum of the frequencies obtained on stimulation of the two sides separately.

Following a testing period of hypothalamic stimulation, the rate of firing of sympathetic neurones to constant hypothalamic stimulation decreases initially and gradually recovers to the control value. This reduction in excitability of sympathetic neurones to hypothalamic stimulation is proportional to the frequency and intensity of the testing stimulation. The duration of depressed excitability is related to frequency, intensity and also duration of the testing stimulation.

The site at which the frequency of firing of a neurone is limited, is caudal to the point of convergence of pathways from the two sides of the hypothalamus, but cephalic to that portion of the system accessible to antidromic excitation.

The pathways connecting the hypothalamus with the frequency limiting mechanism, as well as the more peripheral parts of the system, show relatively less depression of excitability as a result of activity than does the frequency limiting mechanism itself. The suggestion is made that this mechanism lies in the medulla at a point accessible to afferent impulses carried by the buffer nerves.

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